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BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY

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BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY

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SOUTHERN REGIONAL RESEARCH LABORATORY
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COTTON UTILIZATION INVESTIGATIONS

New Cotton Opener in Use by Textile Mills

A textile machine developed at the Southern Laboratory for opening and fluffing up the matted lumps of cotton from the bale before cleaning and carding was mentioned in previous reports. Engineering details of the new machine were released to the industry in September 1950 and as a result it is now in commercial production and use. Six manufacturers of textile equipment are producing the opener. Three are in operation in mills and 5 more are under construction. One manufacturer who purchased two of the machines finds that its use has resulted in large savings. He reported: "We are sure it will pay for itself many times during the first year of operation." The savings will lower the manufacturing costs of textile products. It is estimated that savings up to $1.00 per bale on the lower grades of cottons processed are being obtained.

The new opener, with exceptionally high production for its small size -- it requires only 50 square feet of floor space and has a rated production of 2000 pounds per hour -- is a great aid in cleaning mechanically harvested cottons. The high speed of the opener does not increase the formation of neps, nor affect the grade or strength of yarns. In addition to its value as an aid in cleaning, the machine also reduces the amount of spinnable fiber removed with the extraneous matter by cleaning equipment.

Effect of Mechanical Processing on Fiber Properties is Measured

It cannot be reiterated too often that knowledge of the relations between fiber properties and yarn and fabric qualities is important in research to improve the usefulness of cotton products. Recently, interest has arisen in the significance of the changes which may take place in cotton fibers as they are processed from raw stock into yarn. Any damage that occurred to the fiber during processing would be carried over into the products and affect their appearance or utility.

Investigations have been made in this field at the Southern Laboratory. Studies of the effect of mechanical processing on the individual fiber strength, elongation at breaking point, and viscosity showed that the only significant change in these properties in processed cottons was decreased elasticity. In other experiments, ginned stock from seven commercial American cottons was processed through the yarn stage. Again only the elongation and the modulus of elasticity, which depends upon elongation, and is a measure of toughness, were affected -- the elongation was consistently reduced, and the modulus increased. There was no evidence of damage to the fiber

during spinning. This proof that spinning changes only the elongation properties makes it possible to use treated yarns rather than the unprocessed raw stock in evaluations of the various chemical treatments developed at the Southern Laboratory; and the same yarns used in the processing experiments have now been subjected to these treatments for evaluation.

The changes in mechanical properties of fibers due to heating in the presence of moisture were investigated. Fibers were heated for various periods in atmospheres of different humidities at three temperatures, 110°, 138°, and 162° C. The loss of strength was greater as the moisture content of the atmosphere increased, with the exception that 100 percent moisture -- live steam -- was considerably less destructive, probably because of the absence of oxygen. Data of this kind will be applicable in processes, such as slashing and dyeing, in which yarns or fabrics are subjected to changes in the surrounding atmosphere.

New Data on Chemical Composition of Raw Cotton

Additional data have been needed on the noncellulosic constituents of raw cotton fiber in evaluating the factors that may influence processing behavior in the mill and in finishing operations. The percentages of these constituents have been assumed to vary under the influence of variety, maturity of the fiber, environment of production, and field weathering. As a step toward obtaining the data desired, six samples of commercial raw cottons whose fiber properties had been determined were analyzed for cellulose, total and reducing sugars, nitrogen, wax, ash, ash alkalinity, pH of the water extract, water-extractable material and the several organic acids present. Of particular interest were the variations in the percentages of wax, ash, water-extractables, and organic acids. The percentage of organic acids was found to decrease with increase in extent of field weathering before picking, and with increase in maturity of the fiber.

A small amount of free reducing sugar was found in the water extract of raw cotton. The principal sugars in the extract after acid hydrolysis were galactose and arabinose, but small amounts of rhamnose, inositol, and glucose were also identified. Only glucose previously had been reported to be present.

Evaluation of Hopi Acala 50 -- A High Strength Cotton

The Southern Laboratory is contributing to the evaluation of new and superior varieties of cotton in a coordinated effort among groups which include the National Cotton Council, state experiment stations, and federal agencies. The Southern Laboratory's part of this evaluation program includes determinations of physical properties of fibers, mechanical processing characteristics, responses to chemical treatments, and to mercerizing and dyeing, and small-scale tests of the products in a limited number of selected end uses.

Participating in this program this year, the Southern Laboratory investigated the properties of one of the new high-strength cottons developed by the Bureau of Plant Industry, Soils, and Agricultural Engineering, Hopi Acala 50, a variety which seems particularly suitable for growing in California. To determine whether the high strength had been obtained at the expense of other desirable properties, such as flex life, tire cord manufactured from the new cotton was evaluated. Wheel tests were run on tires made from Hopi Acala 50 cord and from a cord produced from a variety of comparable staple length, Stoneville 2B. The Hopi Acala cotton showed satisfactory flex life as compared to that of the control. Evaluation of the mercerization properties of the new variety showed that it attained a higher degree of luster than Acala 4-42, the variety now grown in California. There was no significant gain in strength by Hopi Acala on mercerization over the gain in the Acala 4-42 variety on mercerization.

The laboratory techniques developed in connection with the evaluation of Hopi Acala 50 will be applied in the evaluation of small lots of inter-species cottons, when experimental lots of these cottons are obtainable.

Re-Use Value Shown for Cotton Fertilizer Bags

Feed and flour bags of dyed and printed cotton cloth have long been used to make dresses, children's clothes, curtains, and slip covers. The market for cotton made possible by this method of merchandising feed suggested to manufacturers the possibility of applying the same plan in the sale of commercial fertilizer. Attractive fertilizer bags could have a thrifty re-use value, too, if they were not damaged by chemical action of the fertilizer with which they were filled, leading to loss of fabric strength or to fading of the colors used, or perhaps to both. Because of the uncertainty, the problem was referred by the Textile Bag Manufacturers Association to the Southern Laboratory.

Representative samples of dyed and printed bag sheeting, as well as a quantity of commercial 5-10-5 fertilizer, were supplied by a member of the Association. Several sets of test bags were made at the Laboratory, filled with fertilizer, and stored under varied atmospheric conditions. One set was kept under conditions approximating those that would normally prevail in a warehouse in New Orleans; one was placed in a hot, dry atmosphere; and a third set was stored in excessively warm, moist air. At regular intervals bags of each color and pattern were withdrawn for examination. At the end of a year there were no significant changes under any of the conditions employed. Men expert in matching dyes could tell no difference in color. Strength losses, by standard laboratory tests, were insignificant. Thus, an indication that colored or printed cotton bags can be packed with commercial fertilizer without appreciable damage to the color or the fabric was provided.

On the basis of these tests the Textile Bag Manufacturers Association and the National Cotton Council have proceeded with their plans to encourage the use of dyed or printed cotton fertilizer bags. Their efforts, according to recent reports, have been attended with considerable success.

Weather-Resistant Tobacco Shade Cloth Produced Commercially

Field trials by Florida tobacco growers of cotton shade cloth given a weather-protective lead chromate treatment by the Southern Laboratory were described in previous reports. The success of these trials has resulted in the commercial production of the treated fabric, which is now available in any desired quantity. One tobacco grower in North Florida purchased in 1951 sufficient yardage of the treated cloth to cover a 10-acre field. Increased durability of the treated cloth is responsible for the interest of tobacco growers. Samples first put on test in 1948 have been used for 3 full seasons as top cover, and are being used a fourth season as wall cover. In contrast, untreated cloth is so rapidly degraded by the action of weather that it serves only one season as top cover and one as wall cover. For this reason, the increasing price of cotton materials made shade cloth such an important item in the tobacco farmer's production costs that the demand arose for protective treatments to prolong its useful life. Cotton shade cloth for tobacco fields costs $500-600 per acre. The lead chromate treatment recommended by the Southern Laboratory more than doubles the useful life but increases the cost by only 10-15 percent.

Advances Made in Testing Fibers, Yarns, and Fabrics

Although the cotton industry has for centuries supplied the world with durable and satisfactory articles in almost every essential civilian and military market, new scientific knowledge is still needed to enhance the efficiency of operation and to improve the quality of products. Cotton growers, merchants, processors, and research workers will be aided in their task of achieving the required improvements by having better testing methods and new information about the significance and the application of the tests. Improved testing methods and equipment speed the work of the breeder and research worker; lower the cost of control methods; provide new information about the relation between fiber properties and yarn and fabric performance; point to the best end uses for specific lots of cotton, thereby giving improved products; and conserve supplies of cotton, a consideration of great importance in the present national emergency.

The following advances made in the testing field by the Southern Laboratory have practical value for many laboratories and mills.

An improved x-ray technique for determining crystallinity. Fiber strength is closely associated with the orientation of the cellulose crystallites in the cotton fiber. For many years scientists have used this crystallite orientation, or the "x-ray angle," as an index of

fiber strength. Such an index is particularly useful in selecting varieties of cotton for laboratory control work and for studies on developing new cotton varieties. The measurement of orientation, unlike the usual mechanical strength tests, may be carried out on a very small tuft of fibers, even those from a single seed. A more rapid method of determining the x-ray angle is provided by the development at the Southern Laboratory of a new rotating specimen mount combined with the use of an x-ray spectrometer. The new procedure eliminates the expensive photographic processes required with other techniques; and, since it traces the x-ray patterns automatically, dispenses with the usual time-consuming densitometer measurements.

Conditioning samples before testing. There has been considerable uncertainty as to whether in testing cotton yarns and cords for breaking strength and elongation the trouble of bringing the test material to the standard conditions, 65 percent relative humidity and 70 degrees F., from the dry or moist condition is necessary to obtain reliable results. The point has been settled in experiments conducted at the Southern Laboratory. Conditioning cotton yarns and cords before their exposure to the standard atmosphere in tests of breaking strength and elongation did not offer a practical advantage, and the expense of preconditioning all samples to approach moisture equilibrium from either the high or the low side appears not to be warranted.

Mathematical expression for fiber circularity. One of the many bases for predicting the behavior of cotton fibers in certain end uses is knowledge of their cross-sectional shape. All cotton fibers are twisted tubes, but the tubes can vary in shape from ribbon-like flatness to almost complete circularity. It is known that the roundness has an important influence on end use characteristics of the fiber or final textile. Fiber shape, for example, bears a relation to maturity, and affects luster, resiliance, suitability for mercerization, breaking strength, and dyeing qualities. But one difficulty in establishing definite correlations has been the lack of a sound, quantitative method for designating shape by means of a simple function which can be used as a numerical index. Now a simple mathematical expression and an instrument have been devised in analytical and cotton-fiber research at the Southern Laboratory to evaluate the circularity of fiber cross sections and to measure the quantities defining the shape. The new technique, which has been used in connection with research to develop better water-resistant cotton fabrics, has general application in all investigations involving a correlation of shape with fiber properties. The instrument and the index in this way will be useful tools in gaining a broader understanding of the relations between individual fiber properties and the useful properties of yarns and fabrics.

Importance of Cotton Waste Shown in Survey

A recent study of the production and utilization of cotton waste in the United States points the way for improving the efficiency of cotton textile processing and utilizing more effectively the different types of waste produced.

This study, conducted jointly by the Southern Laboratory and the National Cotton Council, showed that about 15 percent of all cotton consumed in the United States becomes cotton waste in the process of manufacture in textile mills. Some 70 percent of total cotton waste production is fiber, the remainder consisting of motes, bits of leaf and stem, dust, and other foreign matter. In 1948 United States mills produced 642 million pounds of cotton waste with a value at the mill of $71,000,000.

The results of this study have provided a greater appreciation and knowledge of the size and nature of the waste industry, the factors influencing waste markets, methods for controlling waste, and opportunities for enhancing the value of cotton waste through research. Evidence of the interest in this type of information and of its value as a guide is manifested in the large number of requests received for copies of the report -- about 1,000 since its publication in October 1950.

Fewer Neps Formed Using Improved Techniques of Processing Cotton

Neps are minute, tangled knots of fibers that are formed in cotton during processing. They detract from the appearance of cotton fabrics and lower their quality where appearance is of first importance. These defects are especially undesirable in fabrics which are to be dyed, for the neps take on shades different from those of the body of the fabric.

Methods for substantially reducing the formation of neps during manufacture were discovered in research this year by the Textile School of the North Carolina State College of Agriculture and Engineering in a Research and Marketing Act contract with the United States Department of Agriculture which is being supervised by the Southern Laboratory. Nep formation is influenced by fiber maturity, fineness, and length, as well as by excessive manipulation of the fibers during the opening, picking, and carding processes. It was found that neps can be reduced through careful selection of the raw stock and by making adjustments in the settings and speeds of the opening, picking, and carding equipment. The greatest improvement can be made at the card, by applying methods of distributing the sheet of cotton more evenly on the cylinder and of preventing too rapid loading. Reducing the number of opening processes and reducing the beater speeds or adjusting the rate of cotton fed, to obtain milder treatment of the fibers, also will reduce nep formation.

Some of these recommended measures have been adopted by a number of cotton mills and substantial improvements in yarn quality have been reported.

Luster Characteristics of American Cottons Determined

American cottons can be selected effectively for the manufacture of end products in which maximum luster is desired as a result of work on improving the luster of cotton -- conducted by the Harris Research

Laboratories under a Research and Marketing Act contract with the United States Department of Agriculture which is being supervised by the Southern Laboratory.

In the research it was evident that a first step must be the development of an accurate and dependable means of measuring or characterizing luster in textiles. Since the lustrous appearance of a yarn or fabric depends chiefly on the manner in which light is reflected from its surface, the method which was developed measures mechanically the light-reflecting properties of the cotton textile. It utilizes instruments and techniques usually employed in estimating gloss or reflectance of noncotton materials, such as ceramics. The mechanically determined values of luster in textiles correlate well with the results of visual judgment in a wide range of materials.

A survey was made applying this method to American cottons embracing 103 varieties from 25 growth locations. The measurements showed that significant differences existed between the varieties, but that each variety tended to hold similar rank from location to location in relation to other varieties grown at the same location. Correlation between fiber properties and yarn luster was found to be greatest for the x-ray angle and fiber strength, properties related to internal structure, and to be less marked for fiber length and fineness.

The new equipment, techniques, and knowledge developed in this study should aid further cotton research and breeding work and facilitate the selection of cottons for specific end uses.

SUGARCANE AND CANDY INVESTIGATIONS

Processing of New Sugarcanes

New high-yielding, disease-resistant varieties of sugarcane, and varieties with improved adaptability to mechanical harvesting, are attracting more and more growers, a trend that is making much of the existing information on the processing and milling characteristics of canes generally of less value. It can be assumed from earlier experience that current information cannot be used as a guide to the behavior of the newer canes in respect to the efficiency of juice extraction, the effectiveness of clarification, and the resulting yield of sugar. This has been demonstrated repeatedly in commercial operations, but these operations are on too large a scale to determine the cause. Commercial mills necessarily must handle mixtures of canes of widely different qualities so that it is impossible to study the juice from any particular lot separately.

For this reason arrangements have been made to conduct complete pilot-plant investigations to obtain detailed information on the effects of variations in the properties of the new canes and their juices upon the recovery of sugar. These arrangements include an RMA contract for milling tests by Louisiana State University at Baton Rouge, followed by studies of the subsequent processing steps by the

Southern Laboratory. These studies are conducted jointly in a special pilot plant provided by the University.

Experiments can be carried out on individual samples of as little as two tons of cane, yet processing is continuous and operating conditions are automatically controlled to simulate those of full scale sugar manufacture. In this way it is possible to study the processing behavior of juice from a particular sample of cane, and to correlate results of the experiment with the composition of the cane and juice used. The relatively small amount of sample material required also makes it possible to conduct tests on some of the more promising unreleased varieties of cane to obtain advance information on any peculiarities of composition which may affect ease of processing and the recovery of sugar.

In the program conducted during the 1950 grinding season, two or more experiments were carried out on each of eight varieties of cane supplied by the American Sugar Cane League. These varieties included the newer commercial varieties in Louisiana and two which have not yet been released for commercial planting. The behavior of these cane samples in milling, including the power required for grinding and efficiency of extraction of the sugar, were determined in the milling tests conducted by the staff of Louisiana State University.

Juice obtained from each sample was collected and maintained at a low temperature to permit continued processing at a rate of approximately 50 gallons per hour. In the small scale liming and clarification equipment, the experiments conducted by the Southern Laboratory revealed differences in rates of clarification, volumes of impurities precipitated, and clarity of the juices obtained. In some of the experiments observations were made on the filterability of the precipitate obtained in clarification, on the ease and completeness of crystallization of the sugar, and on the quality of raw sugar produced. These data were correlated with analyses of the original juice and of the sirups and molasses resulting from its processing. The information has been made available by prompt publication to help the operators of Louisiana sugar mills.

Improved Candies for Military Rations

During the past two years the Southern Laboratory, in cooperation with the National Confectioners' Association, has worked closely with the Chicago Food Laboratories of the Quartermaster Food and Container Institute on the development of improved candies for use in military rations.

Confections are needed that combine high concentrations of the various nutritional factors with greatly improved storage life and acceptability to the soldier in the field. A wide variety of candies as nearly as possible like the candy bars and other confections to which the troops were accustomed as civilians is wanted. The problem is to prepare such popularly acceptable candies to meet the storage

and packing requirements of the Quartermaster Corps. The candy must keep safely for six months at 100° F. and two years at ordinary temperatures. Much of the candy for the armed services will be packed in cans, and therefore must be in disc form. The formulas must also be suitable for starch molding, to take advantage of the large capacity of the candy industry for producing molded goods.

Among the improved candies developed for evaluation so far are disc-type fudge, caramel, and a caramel-nougat combination.

A fudge of excellent texture, which should retain its smoothness much longer than standard fudge preparations, is produced by the addition of 10 percent of sorbitol, obtained by the hydrogenation of corn sugar, to prevent drying out. Proportionate adjustments are made of the amounts of sugar and corn sirup used.

The new caramel, which is of such outstanding quality that the Quartermaster considers it particularly desirable for military rations, is the result of experiments with a grade of sweet whey, produced in dry powder form as a byproduct of cheese manufacture. This candy is prepared so that it can be cast in starch and produced in volume in the disc form required for packaging in cans.

The combination disc-form candy, which is very similar in other respects to a high quality candy bar, was made by using a layer of nougat with some of the caramel to which sweet whey was added.

The coating of all these candies is a regular commercial summer-type chocolate which will stand heating to 110° F. for two years, but which does not melt readily when the candy is eaten. Further research is directed toward the development of a chocolate coating of improved eating quality which will also meet the Quartermaster's severe temperature requirement.

Storage tests are in progress to determine whether the high initial quality and acceptability of the candies so far developed will be retained when they are subjected to tests applied to ration items.

SWEETPOTATOES, CUCUMBERS, AND OTHER VEGETABLE UTILIZATION INVESTIGATIONS - SOUTHERN REGION

Research to Aid Improvement of Food Products from Sweetpotatoes

Investigations have been initiated to obtain fundamental information on the enzyme systems and other constituents of sweetpotatoes as related to performance in processing. Information on these biochemical and chemical factors is needed to guide the production of dehydrated and other processed products of improved stability, nutritive value, and consumer acceptability.

Research Continues to Aid the Pickling Industry

During the past year, effective application of information developed cooperatively by the Food Fermentation Laboratory, Raleigh, N. C., and the North Carolina Agricultural Experiment Station enabled a commercial pickle manufacturer to prevent a severe loss from excessive softening of a large lot of brined cucumbers.

Some 15,000 bushels of Palmetto variety cucumbers (produce or slicer type), from the fall crop of 1950 were brined during cold weather under conditions which precluded fermentation. When the cucumbers showed signs of softening within a few days after covering with a 10 percent salt brine, immediate tests were started in cooperation with the processor to find the reason. The results of earlier fundamental investigations by the Laboratory and the North Carolina Station had indicated that the cucumber itself contributes to softening of salt stock by introducing a naturally occurring pectin-splitting enzyme.

This was confirmed in the present instance by examination of samples of the brined cucumbers and brine from 15 vats, along with samples of fresh Palmetto cucumbers from two different producing areas. After identifying the responsible enzyme, the Food Fermentations Laboratory found that it was sensitive to acid and could be destroyed by treatment with either lactic or acetic acid, or inactivated by heating the brined material. Prompt application of these treatments by the manager of the cooperating plant checked the softening before serious losses had occurred, and saved most of the 15,000 bushels of cucumbers which had a brine stock value of about $60,000.

This verification on a commercial scale of the data previously developed in fundamental research on the role of enzymes in the spoilage of cucumbers is another step forward in efforts to improve the processing of food products. The control methods applied in this particular study will be valuable to the entire pickle industry as situations involving losses from the softening of salt-stock cucumbers arise.

Further cooperative work at Raleigh has developed a chart, believed to be the first of its kind, which can be used by cucumber pickle manufacturers to determine the exact amounts of sugar and vinegar needed to put up sweet pickles that will keep safely. Information of this kind has been greatly needed. The procedures formerly used, for the most part, were based on each individual packer's experience over the years. They were far from uniform or reliable, as evidenced by frequent outbreaks of gaseous spoilage -- the result of too little sugar or vinegar, or both.

The chart is based on information obtained in a study of the tolerance to sugar and vinegar of a yeast organism found to be commonly associated with spoilage of sweet pickles in the industry, and on tests in a commercial plant over a 2-year period during which about 40 experimental batches of pickles were made.

Pickles made with the amounts of vinegar and sugar recommended on the chart did not ferment; those made with less of the preservatives underwent gaseous fermentation and spoilage. Laboratory examination of each spoiled lot showed yeasts to be the causitive agent. No doubt this chart, now being prepared for publication, will find widespread use in the pickle industry for standardizing sweet pickle formulae, for reducing spoilage, and for saving sugar.

CITRUS UTILIZATION INVESTIGATIONS - SOUTHERN REGION

Research on utilization of citrus fruit in the Southern Region is conducted at the Citrus Products Laboratory in Winter Haven, Florida, and the Fruit and Vegetable Products Laboratory in Weslaco, Texas.

Conditions for Safe Storage of Citrus Concentrates

Investigations by the Winter Haven Laboratory in cooperation with a commercial processor have determined the permissible limits of temperature and time for storage of frozen citrus concentrates without deterioration in quality. By applying these data the citrus concentrate industry, along with handlers of the product from the time it is produced until it is consumed in homes, can be sure that it is kept safely for the desired, or necessary, period.

Improved Processing of Canned Citrus Juices

During the past year the Winter Haven Station obtained new, basic information on problems involved in the pasteurization of canned citrus juices, leading to improved flavor and keeping quality. One phase of this work has progressed to the point of practical application. Cans filled with juice may expand, or swell, either because the juice was inadequately pasteurized or for other reasons. Serious losses may result unless the exact source of the trouble is found and corrected promptly. This can be accomplished by applying a test developed at the Station for determining the effectiveness of the pasteurization treatment. When the juice is known to be properly pasteurized, it is a simple matter to track down the trouble elsewhere. Both citrus processors and can manufacturers have shown a great deal of interest in this test, termed an index to pasteurization, and it is proving extremely valuable as a control measure in canning juices.

Other studies, in a less advanced stage, show a definite relationship between the extent of pasteurization and the keeping quality of canned orange juice.

Utilization of Molasses-Added Citrus Pulp Improved

Addition of some citrus molasses to the byproduct citrus pulp improves the palatability and nutritive value of the feed and extends the market outlet for these byproducts. As the practice expanded, however, some questions developed in the industry as to how the

addition of molasses would affect the keeping quality of the pulp. In addition, there was need for a rapid method for determining the soluble solids content of the molasses-pulp feeds. Such information is essential to the formation of trading standards, in which the industry became interested as the supply of byproducts increased with the growth of the citrus processing industry during recent years.

Investigations undertaken by the Winter Haven Laboratory to answer these questions indicated that the addition of molasses did not introduce a storage problem. Although there was some increase in the absorption of moisture when molasses was added, the effect was much less than that of the relative humidity of the air in which the product was stored. Neither plain nor molasses pulp became sufficiently moist at 70 percent relative humidity to support the growth of molds, but both became moldy at 80 percent. By drying the molasses with the pulp, practically all danger of spoilage from molds or heating can be eliminated.

A method developed by the Laboratory for estimating the soluble solids in dried citrus pulp feeds is being used to advantage by the industry as an aid to improving the uniformity of the feeds and in establishing satisfactory standards for their manufacture and marketing.

During the past year an expanded program of research on the processing of Texas citrus fruit was inaugurated at the Weslaco Laboratory in cooperation with the Texas Citrus Commission, the Texas Agricultural Experiment Station, Rio Farms, and the Bureau of Plant Industry, Soils and Agricultural Engineering. Investigations, under this program, on preservation of single strength citrus juices and production of improved frozen concentrates were severely hampered by the severe freezes of early December and last January. The impaired quality of most of the available fruit made the results of the limited experiments that could be conducted inconclusive.

The stability of the color of red grapefruit under normal conditions of low temperature concentration and storage was again demonstrated this season. Progress has been made in establishing the nature of the color, but final identification of the pigment has not been completed. Definite identification will facilitate development of means to stabilize the color in processing the juice, which now becomes brown or muddy on pasteurization and canning.

RICE UTILIZATION INVESTIGATIONS - SOUTHERN REGION

Effect of Heat on the Germination of Rough Rice

Investigations in cooperation with the Rice Experiment Station at Crowley, Louisiana, have defined the conditions of heating which do not interfere with germination over ranges of moisture content from approximately 5 to 25 percent, temperatures from 45 to 75° C., and heating periods from 10 to 120 minutes. The information will be applied to control of drying conditions, destruction of plant pathogens, improvement

of milling yields, and development of treatments to improve storage.

Anaerobic Storage of Rough Rice

Data have been obtained from two experiments conducted by the Crowley Station on storage of rough rice in air-tight sealed bins. It appears that such anaerobic storage prevents heating, but promotes development of yeast-like organisms and imparts an undesirable odor to the grain. After drying, the rice milled well but the product had a gray color and a noticeably sour odor.

OILSEED UTILIZATION INVESTIGATIONS

Advances Made in Coordinated Research on Cottonseed Meals of Improved Nutritive Value

In five cooperative processing experiments at two commercial oil mills during the past year, 12 tons of cottonseed meals of improved nutritive value were produced with two types of screw presses. Quantities of the meals thus prepared were sent to nutrition investigators in the Bureaus of Animal Industry, Dairy Industry, and Human Nutrition and Home Economics of the USDA; the Arkansas, Florida, South Carolina, and Texas Agricultural Experiment Stations; and a farmers cooperative (Eastern States Farmers Exchange) for conduct of experiments to evaluate the nutritive quality. The meals furnished reflected the effects of several variations in the processing conditions: maximum temperature of cooking; energy input to the motor driving the press; and rate of throughput of the press. All had a relatively low content of free gossypol; but there was considerable variation in the nitrogen solubility and thiamin content with different lots. Samples of screw-press meals from regular commercial operation were included for comparison with the experimentally-produced meals.

At a conference held at the Southern Laboratory in November, 1950, nutrition workers from State experiment stations of the Southern Region and from other Bureaus of the USDA reported on the results of their experiments with earlier series of meals produced experimentally at the Southern Laboratory and in a commercial mill. Meals produced with a moderate maximum cooking temperature of around 200° F. had a higher protein value than did those cooked to a higher temperature and much higher than some screw-press meals which had been cooked at temperatures as high as 270° F.

Experimental screw-press meals of low free gossypol content fed to hogs in concentrations as high as 43% of the total diet supported good growth and there was no gross evidence of toxicity. Similarly the meals were fed to chicks up to 70% of the diet without any harmful effect and with good support of growth. Heretofore recommendations for the use of cottonseed meal in such feeds have called for concentrations not to exceed 10 percent for hogs and as little as 5 percent for chickens. At two different State experiment stations it was shown that meals of low free gossypol content furnished by the Southern Laboratory did not produce egg yolk discoloration when fed to hens at levels of 20 to 25% of the diet. On the basis of present information a content of .03% of free gossypol appears to be the highest that can

be tolerated by chicks and swine fed unlimited quantities of cottonseed meal; and meal containing .01% of free gossypol can be fed to hens without interferring with egg yolk coloration. The exact levels in either case will be determined by experiments currently under way.

The results of research on solvents to reduce the free-gossypol content of cottonseed meal to a minimum without resorting to overheating have been applied in the pilot-plant production of quantities of meal of low gossypol content and high protein value for use in nutritional experiments as a standard of comparison.

With more and more investigators giving serious consideration to the effect of processing variables on the value of the product, an entirely new approach to research on the utilization of cottonseed meal is now under way. Heretofore, nutrition investigators have had the complete responsibility of obtaining the meals, having them analyzed, and determining their nutritional properties. Under such an agreement it was difficult to give sufficient attention to the effect of processing variables.

Accordingly, the Southern Laboratory has developed, in cooperation with the National Cottonseed Products Association, a new improved arrangement for nutrition research which provides for the cooperation of all parties involved from the production of the meal to its nutritional evaluation. Cottonseed meal is produced in commercial mills under conditions specified by the Southern Laboratory. These meals are analyzed and characterized at the Laboratory and then are furnished to nutrition investigators in state, federal, and commercial laboratories. The arrangement makes it possible to relate the results of feeding tests with the chemical analysis and processing history of the meals. Systematic changes can be made in processing conditions so as to study the effect to better advantage. In the last three years, 40 tons of experimentally produced cottonseed meals have been supplied for nutrition research under this program.

This coordinated program of research on cottonseed meals, with emphasis on processing history as related to nutrition, is paying off with information which promises to greatly increase the production of high quality cottonseed meal. Since the need for protein supplements for feeds and foods is unusually great in emergency periods, the procurement of new knowledge on cottonseed meal is now particularly timely.

Usefulness of Cottonseed Meal Glues Established

Earlier tests on the use of glues made with cottonseed meal were extended from a laboratory to an actual-service basis. In these service tests, conducted under accelerated conditions, plywood panels bonded with the glue were first subjected to an "interior severe" test, during which they were kept 48 hours under warm, humid conditions (80° F. and 80 to 85 percent relative humidity). When they passed this test the panels were allowed to come into equilibrium with an atmosphere of 32 percent relative humidity and temperatures between 80° and 85° F. They were then given an "exterior" test which involved

immersion in water at 75° F. for 48 hours. These tests were repeated until the glue failed to hold the panels together.

The results showed that cottonseed meal glues are entirely satisfactory for bonding plywood that is intended primarily for interior use in regions where the average relative humidity is low. Where the humidity is high, cottonseed meal glues might not produce a sufficiently permanent bond for satisfactory use. Added to the information on hand, these service tests provide all the data needed by industry in judging the potential usefulness of this new cottonseed product.

Glues prepared from meals obtained by solvent extraction, where a minimum of heating is involved, gave stronger bonds than those prepared from meals obtained by other processes. Meals from commercial solvent-extraction operations were satisfactory.

Improved Process for Producing Cottonseed Oils of Light Color

Many cottonseed oils produced in several areas of the cotton belt, particularly those produced by continuous or screw pressing, when refined by conventional methods, fail to yield oils of acceptable color and quality for use in the manufacture of shortening. Such oils are normally subjected to a second or re-refining. Even after re-refining they still may not meet specifications for a shortening oil of the very light color required by the trade.

Experiments performed by the Southern Laboratory have demonstrated that acceptable products can be made from these off-colored oils by modification of the conventional re-refining process to use increased concentrations of caustic soda solution and very high rates of agitation and shear in mixing the oil and alkali, within certain specified temperature limits.

The reduction of the color of refined cottonseed oil was found to increase as the rate of agitation and shear of re-refining increased up to an extremely high rate beyond which further increases had little or no effect. About 0.2 percent of caustic soda (on an oil-weight basis) used in the form of a 14 to 24 percent solution, time of agitation between 5 and 10 minutes, and a maximum temperature of 150° F., were found to produce optimum reduction in color of the oil. The color of the oils re-refined by the high shear method usually was half or less of that obtainable in the same oils by conventional re-refining. This proportional difference in color was also observed in the oils after bleaching. The high-shear re-refining method removed the acidic gossypol pigments of cottonseed oil more effectively than did conventional re-refining. In addition, the new method removes a high percentage of unidentified red pigments unaffected in conventional re-refining. The changes from the conventional process, while simple, are radical, since the conditions found to be most effective for removing the color were previously believed to be deleterious to the oil and represented unacceptable refinery practice. Aside from the fact that it requires a small amount of special mixing equipment and consumes more power than does

conventional re-refining, the new process has no known disadvantage. Conventional materials are employed, the oil requires no special treatment before or after re-refining, and re-refining losses are the same as those encountered with the conventional process.

Antioxidants Tested in Cottonseed Oil

During recent years a number of chemical compounds with properties indicating that they would be useful in stabilizing fats and oils against oxidative deterioration, or rancidity, have become available. The majority of these compounds are phenols of widely different chemical structures -- some relatively simple, others quite complex. On some, there has been considerable information on which to base their use in certain fields, but usually not enough to be a reliable guide to their effectiveness as antioxidants. In some cases the information was meager, and data for evaluating the overall usefulness of these new products were quite inadequate.

As part of its program of research to improve the utilization of vegetable oils from Southern-grown oilseeds, the Southern Laboratory has conducted an investigation aimed specifically at providing data for comparing the effectiveness of different antioxidants as a means of stabilizing cottonseed oil against rancidity.

In previous research the laboratory had demonstrated that cottonseed oil, when carefully processed and handled, has a high inherent stability toward oxidative rancidification because of certain natural substances (tocopherols) that are present. Hydrogenation, the process used in preparing the oils for use in shortenings and margarines, further improves the natural keeping quality. Nevertheless, additional improvements are desirable. Thirteen antioxidants were tested for their ability to improve the keeping quality of a finished edible cottonseed oil and the same oil hydrogenated to shortening consistency. For comparison these same antioxidants were added to prime steam lard, a product which is relatively low in natural antioxidants. As might be expected they were much more effective in stabilizing the keeping quality of the lard than they were for either of the oils. Propyl gallate was found to be the best of the antioxidants tested for both hydrogenated and unhydrogenated cottonseed oil. While less effective than propyl gallate, nordihydroguariaretic acid and norconidendrin were found to be good antioxidants. Most of the other ten antioxidants tested gave little or no increased protection to cottonseed oils although they were effective with lard even in small concentrations.

Norconidendrin was prepared in the laboratory by altering the chemical structure of conidendrin, a compound recovered from the waste sulfite liquor produced during the pulping of western hemlock. On the basis of the laboratory's work a private concern has recently undertaken limited commercial production of norconidendrin. Its effectiveness as an antioxidant has been established in a number of important industrial applications besides stabilizing vegetable or animal oils and fats. Because of its possibilities in the food and

food-packaging fields, studies have been undertaken at the Western Regional Research Laboratory to ascertain whether or not norconidendrin can be used safely in edible products.

The study of antioxidants completes a thorough investigation in which much new and valuable information on the stability of cottonseed oil has been provided during recent years. Of particular significance are data explaining the mechanism by which fats and oils absorb oxygen and become rancid. Working first with the simple unsaturated fatty acid ester, methyl oleate, and later with cottonseed oil, the reaction of atmospheric oxygen with these substances was found to produce hydroperoxides which on decomposition produced various saturated and unsaturated aldehydes that impart rancid odors and flavors to the fat or oil. This knowledge, obtained in work on cottonseed oil, applies also to peanut oil, and has been extremely valuable in developing the additional knowledge that is now available generally to improve the keeping quality of fats and oils.

New Flexible Coating Fats

There has long existed a need for an edible, highly flexible, non-greasy fatty material to replace paraffin and other non-fat coatings presently used in the food industry. In general, flexibility and lack of greasiness have been mutually exclusive properties of fats. A non-greasy fat is usually hard and brittle whereas a flexible fat is usually soft and oily.

Recently, however, the Southern Laboratory has developed flexible and non-greasy fats that can be prepared in a fairly simple manner from monostearin, a monoglyceride prepared from glycerine and completely hydrogenated cottonseed oil or a similar fat, and acetic anhydride, an inexpensive commercial chemical made from acetic acid.

A variety of acetostearins can be produced with melting points ranging from 80° to 140° F., depending upon the grade of monostearin used and the degree to which it is permitted to react with the acetic anhydride. Generally, such products melt over a temperature interval of 2° to 5° F.

Below their melting points and down to room temperature most acetostearin products are non-greasy and flexible. Below room temperature, the majority of the experimentally prepared acetostearins could be stretched over 800 percent without breaking. Even the least flexible acetostearin product stretched six times as much as did paraffin wax (melting point, 125° F.). At freezing temperatures the acetostearin products are still quite flexible.

Although there are no known facts to indicate that these flexible fats are not edible and digestible, they are now being carefully evaluated in this respect by several laboratories. Their use as coatings for cheese, dressed meats and poultry, ice cream bars, and other food products is being explored, along with interesting possibilities in non-food fields.

Quality of Peanut Butter Improved by Control of Roasting Conditions

In continued research to determine the exact effect of processing on the quality of peanut butter, products prepared from peanuts roasted over a wide variety of conditions and stored at 80° F. for as long as 21 months have been evaluated both chemically and organoleptically at the Southern Regional Research Laboratory.

These evaluations, together with the results of previous laboratory and pilot-plant studies, show definitely that careful control of roasting conditions is necessary for the production of peanut butter of optimum flavor and good keeping quality, and furthermore that the range in desirable roasting conditions is rather narrow.

New data also support previous observations that the oil contained in peanut butter is relatively stable to oxidative rancidity. Oils obtained from fresh peanut butters were found to have low peroxide values and long keeping times. Although some reduction occurred for oils extracted from stored peanut butters during the first three months, the keeping times were still relatively long. No appreciable differences were found between the keeping times of oils from butters after storage for three months and those from butters after extended storage.

Taste panel tests indicated a preference for medium-roasted butters stabilized with hydrogenated peanut oil for the prevention of oil separation, the average scores for such products being higher than those given to lighter colored butters. In general, peanut butters stored at 80° F. and examined periodically by the panel were found to retain acceptable odor and flavor characteristics for as long as a year, after which the products developed objectionable flavors before any appreciable oxidative rancidity could be detected in the extracted oils. A definite tendency was noted for the butters made from the heavier-roasted peanuts to receive unsatisfactory flavor ratings at an earlier age than the butters made from the lighter- and medium-roasted peanuts.

Such information is of considerable value to the peanut butter industry in selecting the conditions of processing that will produce products with a high consumer rating and long shelf life. This information is of interest to the Quartermaster Corps because of its value in assuring the production of peanut foods of high quality for defense personnel.

Research Aids Expansion of Rice Bran Oil Industry

The production of rice bran oil as a byproduct of the rice milling industry has grown rapidly in the United States during the past few years, largely as a result of technical information made available by the Southern Laboratory. Several plants operating at full capacity in 1950 put large quantities of rice bran oil on the market; but the total output was only a fraction of the 50 million pounds, with a potential value of $10,000,000, that could have been produced from the year's rice crop. A new plant is under construction in 1951.

In further investigation of the rapid deterioration of the oil in rice bran, with development of a high content of free fatty acids, when stored between milling and extraction, evidence has been obtained that the rate at which these free fatty acids form is highly dependent upon the moisture content of the bran. However, the biological factors responsible for the deterioration occur in the bran itself or in the microorganisms found on the bran. Information is not yet available on the effect of the enzyme systems of the bran itself; but experiments on sterile bran inoculated with selected strains of mold and bacteria demonstrated that these agents can cause the development of free fatty acids in the bran oil and, therefore, must be controlled in order to completely prevent deterioration during storage. It was observed further that moisture hastens the growth of both molds and bacteria.

The obvious conclusion is that rice bran should be stored in as dry a condition as possible. While the moderate heating normally used to dry rice bran was found to destroy very few of the microorganisms present, and apparently did not affect the enzyme systems at all, such heating did remove water which permitted the hydrolytic reactions involved in the formation of free fatty acids. Thus, if bran dried in this manner could remain dry, deterioration would be prevented. However, whenever the dry bran was again exposed to the high humidities prevailing in the southern rice-growing areas it became rehydrated, permitting the natural systems of the bran and the oil microorganisms to promote its deterioration.

Autoclaving the bran, on the other hand, effectively sterilized it free of microorganisms and destroyed the natural tendency for deterioration. Regardless of moisture content, free fatty acids will not form in bran which has been sterilized by autoclaving and then maintained in a sterile condition. Sterilization followed by dehydration, therefore, would seem to be a method for complete control of deterioration during the period when rice bran must be stored prior to solvent extraction. Even if the bran partially rehydrates during this period, the onset of deterioration will be slow. This information should be of practical value to the industry in developing means of storing rice bran without deterioration.

In another line of research information has been obtained to improve techniques for refining rice bran oil. In the past, there have been difficulties especially when the oils were high in free fatty acid content, as they often were if the bran had been stored for any length of time prior to extraction. An experimental procedure that involves steam-stripping the oils seems promising as a solution to this problem. The crude rice bran oil is bleached, degummed, and dewaxed in the usual manner, then is treated with steam before the usual refining st p where an alkali is used. In pilot plant experiments steam-strip ing reduced the free fatty acid content of rice bran oil from about 9 percent to less than 1 percent and lowered refining losses sufficiently to yield 8.5 percent more oil. Additional studies are under way to determine the best conditions (temperature, vacuum, amount of steam, and so on) for practical application of this procedure.

Analysis of samples of bran from eight commercial varieties of rice, each of which was grown in Texas, Louisiana, and Arkansas, showed that the purified bran (the true pericarp and germ fraction) contained from 18 to 25 percent oil, depending more on the variety of rice than on the environment of production. The amount of oil in commercial bran is lower, of course, since the milling operation removes some of the inner part of the grain (endosperm) along with the bran. Variations in milling, as well as in physical condition of the grain, affect the amount of oil.

Safe Moisture Content for Storage of Tung Fruit

Investigations at the Tung Oil Laboratory, Bogalusa, Louisiana, have provided new information on the water-absorbing and water-holding properties of tung fruit which is proving valuable in overcoming problems of proper drying of the fruit and seeds to insure safe storage and efficient milling.

With a moisture content of about 65 percent as it falls from the tree, tung fruit will heat, mold, and sprout if stored before it has dried to about 25 percent moisture. Even under favorable weather conditions, this takes several weeks, and sometimes in wet seasons the fruit never will dry sufficiently on the ground to permit storage. Artificial drying of the whole fruit, on the other hand, is not economical. Since the hulls contain more than half the total moisture present in the whole fruit and have no value as a source of oil, their removal before drying or storage is desirable to decrease the heat required for drying and also to reduce the space needed for storage. However, hulling followed by the usual storage procedures has some disadvantages that are discouraging to growers. Seed broken in the hulling operation quickly spoil if stored in their normal moist condition, develop free fatty acids rapidly, and sometimes heat.

It was found that tung fruit and its products tend to assume a moisture content in equilibrium with that of the surrounding atmosphere. In contact with the atmosphere at 75 percent relative humidity, which is average for the tung area, the equilibrium moisture contents for tung fruit, kernels, and seed are about 12.4, 6.1, and 8.2 percent, respectively. If the products, then, are dried to these moistures, they can be stored safely and processed efficiently. It is particularly significant that the hulls can be removed and the seed dried to 8 percent moisture with assurance that they will not spoil in storage. In recent work the Laboratory has extended these investigations to determine safe storage conditions for tung hulls and press cake, which frequently spoil as a result of spontaneous heating.

NAVAL STORES INVESTIGATIONS

During the past year the fundamental research of the Naval Stores Research Division, which had been conducted at the Southern Laboratory in New Orleans, was transferred to the Naval Stores Station at Olustee, Florida, and now all research of the Division is conducted at that location.

Continuous Distillation of Cleaned Pine Gum

The 32-inch commercial size continuous steam still developed at the Naval Stores Station, Olustee, Florida, was operated successfully at its full capacity of 10,000 pounds of cleaned pine gum per hour. The capacity of this still is equal to that of the large batch stills in the industry and is believed to be the maximum desired by any processor of pine gum. The rosin produced in typical continuous runs with the new still had a satisfactory melting point and a low content of volatile oil, and the turpentine had a low acid number. To realize the maximum benefits of continuous distillation with this equipment, commercial plants would operate on a 24-hour work day. Continuous distillation would result in a 50 percent saving in steam consumption, lowered labor and other operating costs, high-quality rosin and turpentine, and less variability in product quality. Completion of the new still ends nearly 10 years of process development research.

Complete engineering data will be furnished, upon request, by the Naval Stores Research Division in Olustee, Florida.

Improved Practices in Gum Cleaning

Continuing to help processors who use the Department's public service patents on gum cleaning meet the demand for quality products, engineers of the Naval Stores Station this year supplied details on improved practices of filtration, washing, and handling of chips.

The filtration of crude pine gum by the gum naval stores industry is a phase of plant operation in which savings in labor, time, and material costs can be of great value. To obtain these improvements, the Naval Stores Research Station, in cooperation with a filter manufacturer, modified a commercial self-cleaning filter to adapt it to pine gum processing. The new-type filter requires only the part-time services of one man for operation, uses more economical filter media, and can handle the low-quality gum that gives trouble in the filters generally used by the industry. The capacity of the filter, comparable to that of the conventional types, is such that only one cleaning per day is normally required. The first commercial installation of the new self-cleaning filter has been made.

The attention of processors was called to the necessity for the thorough washing of gum during cleaning to produce high-quality rosin. Although a single washing in the Olustee process produces high-grade, brilliant rosins, such rosins may still contain traces of contaminating materials. A thoroughly clean rosin can be made by recirculation of the filtered, washed gum a second time through the wash tank.

A third simple step in cleaning operations, recommended to processors as a means of upgrading their pale rosin when there is a price differential in favor of the paler grades, is the following: Separate the chip cleanings from the main charge and distill them with a lower grade of gum. This slight but important change in the regular procedure

will raise rosin grades and allow processors to count on larger quantities of pale-grade rosin. Also, the chip cleanings from WG grade gum, if distilled separately from the original gum, will produce I grade rosin.

Peroxides from Turpentine

The production of perxodies from turpentine and the potentialities of these peroxides as replacements for the conventional peroxide catalysts in making synthetic rubber have been reported previously. This development has defense significance since the usual catalysts are based on benzene, which is in short supply. On the other hand the production of gum turpentine in 1950-51 was 264,000 barrels, only a small part of which went into industrial uses. Replacement of the benzene derivatives by terpene peroxides in the manufacture of about 700,000 tons of synthetic rubber would consume about 50,000 barrels of turpentine. This amount of turpentine would represent a considerably larger percentage than has previously gone into industrial uses.

The Akron Laboratories of the Office of Rubber Reserve last year in laboratory-scale tests demonstrated the superiority of the terpene peroxide, pinane hydroperoxide, to cumene hydroperoxide, which was obtained from benzene. These Laboratories have now confirmed this superiority on a pilot-plant scale. They prepared and tested approximately 300 pounds of "cold rubber" (synthetic rubber made at relatively low temperature) using pinane hydroperoxide produced by the Naval Stores Research Division. They reported that the reaction rates and maximum conversion obtained with pinane hydroperoxide were considerably higher than those realized with the same quantity of cumene hydroperoxide at each of the levels investigated. The properties of the rubber made with terpene hydroperoxide as the catalyst were equivalent to those of similar stocks made with cumene hydroperoxide. Commercial production and use of the terpene peroxide, which appears feasible, would have the advantage under current conditions of conserving benzene supplies.

WESTERN REGION
M. J. Copley, Director

CEREAL AND FORAGE CROPS UTILIZATION INVESTIGATIONS

In the Western Region, work under this classification has been done on three major crops: alfalfa, wheat, and rice. In addition to the work reported in more detail below, the following projects might be mentioned. The research on alfalfa has included spectrographic determination of metallic constituents, growth regulating and inhibiting factors, and other chemical constituents. For wheat, additional basic work has been done on the chemistry of gluten and on the soluble non-gluten, non-starch constituents. On rice, the major emphasis is on the development of a more satisfactory quick-cooking rice and on frozen cooked rice. Other work on this cereal has been on ways for improving the storage stability of brown rice, and on analytical methods for quickly determining the stability of rice bran or brown rice.

Stabilizing Carotene in Alfalfa Meal

Of 300 compounds tested at the Western Regional Research Laboratory for effectiveness in stabilizing carotene (provitamin A) in dehydrated alfalfa meal, two are undergoing field testing in collaboration with industry. These are a hydroquinone and a dihydroquinoline (2,5-ditertiary butyl hydroquinone and 6-ethoxy-2,2,4-trimethyl-1,2-dehydroquinoline). Patent coverage for the latter for this purpose has been obtained. Synthesis of a series of new compounds by replacement of the 6-ethoxy substituent with others did not reveal superiority in carotene stabilization.

Antioxidants are usually applied to alfalfa with vegetable oil (soya, cottonseed or rice bran oil) as a carrier agent. The dihydroquinoline compounds are highly soluble in vegetable oils, so that it is possible to apply them in effective concentrations with the minimum amount of oil required for dust abatement (about 8 pounds of oil per ton of alfalfa, or 0.4 percent). The 2,5-ditertiary butyl hydroquinone and N,N'-diphenyl-p-phenylene diamine (the latter is used commercially in alfalfa) require about 20 pounds of vegetable oil per ton of alfalfa (1.0 percent) to dissolve enough antioxidant to give a final concentration of 0.015 percent when applied to the meal.

Application of the more effective antioxidants to dehydrated alfalfa is being studied with the Poultry Producers of Central California, a cooperative, at their Ryer Island, Calif., plant. The compounds are applied in suitable carriers under full-scale industrial conditions. The application can be made with relatively simple equipment. When alfalfa was treated with 0.015 percent of the dihydroquinoline in 1 percent cottonseed oil and stored six months at room temperature it retained 58 percent of the original carotene while the untreated material was reduced to 31 percent. Alfalfa meal treated with the hydroquinone derivative retained 45 percent of its carotene under like conditions.

Before antioxidants can be used on a feedstuff they must be shown to be satisfactory from the standpoint of toxicity, both acute and chronic. Toxicity tests with rats on the dihydroquinoline and the hydroquinone are in progress in the Pharmacology Laboratory of this Bureau. Similar studies with chicks are being conducted cooperatively by the Colorado Agricultural Experiment Station, the American Dehydrators Association, and the Western Regional Research Laboratory. Under this agreement funds furnished to the Colorado Station by the American Dehydrators Association defray the major expense while the Western Laboratory supplies the test materials and makes final chemical evaluation of animal tissues. Tests thus far have shown the acute toxicity of both compounds for rats to be low, and no significant toxic symptoms have been observed in rats fed the hydroquinone at 0.2 percent level over a period of a year. This is 260 times more antioxidant than would normally be present in prepared feed. Similarly encouraging results are being obtained with the dihydroquinoline.

Determination of Milling Quality with Small Samples of Wheat

In wheat breeding programs the large samples necessary for the determination of milling and baking quality entail extra years of growth of new wheats and thus slow down the program in general. Consequently a coordinated effort to identify the factors responsible for differences in milling characteristics is being made by the Western Wheat Quality Laboratory, Bureau of Plant Industry, Soils, and Agricultural Engineering, Pullman, Washington, and the Western and Northern Regional Research Laboratories. The Western Wheat Quality Laboratory has collected samples of known variety and history, obtained milling data, and supplied samples and milling data to the other two laboratories. The Western Regional Laboratory is relating compositional differences to milling behavior. The Northern Regional Laboratory is determining whether structural differences of importance in the milling operation can be found.

Work in the Western Regional Laboratory has suggested that a crude-fat-to-crude-fiber ratio above 1.0 in a whole wheat indicates that type of poor milling behavior characteristic of Rex variety. Further analyses on 1950 crop samples were consistent with the previous results. The fat-fiber ratio test was suggested by the possibility that a relatively high fat content contributed to the difficulty in sifting Rex flour and mill streams by causing flour particles to clump.

Milling behavior of wheats is however too complex to be expected to depend upon one factor. Because the fat-fiber ratio does not differentiate certain varieties (Baart, Orfed, Brevor), that are intermediate in milling properties, from the best milling varieties (Elgin, Hymar), other factors must be involved.

The endosperm of poor-milling wheats has been reported to fracture in a random manner upon application of pressure, while that of good-milling wheats fractures along cell-wall lines. To determine where in the milling process these or other differences in endosperm properties might affect milling behavior, the bran of Elgin and Brevor samples has been removed by a sulfuric acid-water treatment. The milling behavior of these "debranned" samples then was determined on a micro mill by BPISAE workers. Certain characteristic differences--in release of middlings and rate of reduction of middlings--were still present; consequently these differences in milling properties must be attributable to differences in endosperm, rather than bran, of these varieties. In exploratory experiments, analyses for various hemi-cellulose fractions which are characteristically associated with plant cell walls have been made. The results suggest that the amounts of certain pentosan fractions may be useful in predicting endosperm milling properties; and further investigation may provide more precise methods for indicating milling behavior of small samples.

COTTON AND OTHER FIBER CROPS UTILIZATION INVESTIGATIONS

In addition to the felting studies on wool reported below, work is underway on finding improved methods for scouring grease wool through fundamental studies on detergent action.

Modification of Wool to Improve Felting

Studies on chemical modification of wool at the Western Regional Research Laboratory have shown that treatment with beta-propiolactone results in markedly enhanced felting power.

The ability of wool fibers to felt is a distinguishing and useful property. During finishing, woolen fabrics are subjected to felting conditions ("fulling") to impart desirable body to the material. Manufacture of wool felt is an industrial activity that produces annually about 25 million pounds of products, excluding woven felts, having a value of 20 to 30 million dollars. In the manufacture of felts about 10 pounds of new wool are used per pound of reprocessed wool. Wool felts are used as oil retainers, wicks, sound insulators, vibration mountings, gaskets, etc.

The new derivative is made by treatment of wool with propiolactone, which has recently become available commercially. Laboratory studies of propiolactone-modified wool have shown that both rate and degree of felting are increased. Commercial-scale runs at two felting plants have confirmed the fact that modified wool exhibits markedly improved felting properties. Felts prepared from treated wool are soft and pliable when wet and become extremely hard on drying. In addition, split resistance and tensile strength are significantly higher. Increase in felting power is also found in blends of treated wool and commercial felting stocks.

Laboratory tests have indicated that wool flannel cloth also felts more readily after modification with propiolactone, as indicated by hand-milling shrinkage tests. For both loose wool and cloth, the tests show that felting ability of modified wool varies

with extent of treatment and passes through a maximum at about 50 to 60 percent of propiolactone uptake. More extensive treatments impart shrink-resistance to wool flannel cloth. Mohair modified with propiolactone also shows greater felting ability.

A considerable number of other chemical modifications have been investigated with regard to effect on felting, but none has been found to increase rate or degree of felting; these results indicate a unique character for the propiolactone treatment.

Propiolactone modification may also prove valuable for the treatment of artificially crimped wool and mohair. Artificially crimped fibers have been shown to have enhanced weaving properties and abrasion resistance, but they suffer from low breaking elongation. subsequent treatment with propiolactone tends to recover the characteristic high breaking elongation without destruction of added desirable properties.

Propiolactone modification is comparatively easy to carry out. The conditions have been investigated with respect to solvent, lactone concentration, prior drying of wool, pH, fineness of wool, temperature, etc. A satisfactory procedure is treatment of air-dry wool with a 3 percent solution of propiolactone in carbon tetrachloride with a lactone-to-wool ratio of about 3 to 1. Additional laboratory investigation is required before the propiolactone treatment can be recommended for commercial adoption.

Studies on Electrical Properties of Wool at Microwave Frequencies

Radar equipment developed for use in World War II has been used at the Western Regional Research Laboratory in studies of such electrical properties of wool as dielectric constant and dielectric loss in the microwave region. Work with fibers in this previously uninvestigated portion of the spectrum has yielded clues regarding the structure of wool. Until recently it was not practical to use such methods because sources of microwave radiation were not available. For the fiber work, modified forms of radar equipment were used. In addition, a special apparatus called a cavity resonator was developed. This apparatus consists essentially of a slotted cavity resonator and a frame to support a bundle of wool fibers.

The microwave region is of special interest for fibers such as wool, for which the dielectric constant is large at the highest radio-frequencies. Wool apparently contains a polar unit small enough to follow the rapid alternations of radio-frequency fields. In order to characterize or identify the part of the wool molecule responsible for this behavior, it is necessary to make electrical measurements at frequencies in the range where the polarizable group fails to follow the alternations of the electric field. Results show that at a microwave frequency of 3,000 megacycles per second, the polarizable group is able to follow the alternations almost as well as it does at one one-thousandth of this frequency. This indicates that the group responsible for the high value of the dielectric constant in the microwave region is not an ordinary dipole. Its behavior may be due to the hydrogen bonds which hold adjacent protein chains together.

The new equipment is being used also to investigate molecular interaction between water and wool. As is well known, small amounts of absorbed water markedly influence the physical properties of wool. In addition, a number of instruments used in the textile industry make use of dielectric properties to measure moisture content of fibers, yarns, and fabrics. The new work at microwave frequencies has been especially informative because at these frequencies the dielectric constant of water undergoes a twenty-fold change. Thus the dielectric constant of the wool-water system at microwave frequencies becomes a very sensitive index of the interaction between wool and water molecules. Results obtained show that from 0 to about 10 percent moisture, the percentage change in dielectric constant of wool is larger than at frequencies in the radio range. Since the microwave measurements are simpler to perform and are probably more reliable than measurements in the radio-frequency range, the new results may prove to be the basis for the design of an improved moisture meter.

FRUIT AND VEGETABLE UTILIZATION INVESTIGATIONS

The importance of fruit and vegetables in the Western Region accounts for the fact that a large share of the Bureau's program in this area is on these commodities. In addition to the projects described, other topics have received attention during the past year: a comparison of costs in various methods of supplying apples for pie stock; additional improvements on the wax-dipping process for preventing mold growth on berry hallocks (boxes); processing of frozen strawberries; dehydrofreezing of prunes and carrots and additional work towards improving the dehydrofreezing process including studies on the kind of equipment needed for commercial operations; analytical and physical-chemical investigations, including X-ray diffraction patterns, optical characterization of hydrates of sucrose and levulose, measurement of color, volatile constituents of peas and of tomato juice, and phase equilibria of sugar solutions; additional work on vitamin B_{12} production; effect of storage temperature on quality of frozen peas; enzyme inactivation in frozen lima beans; discoloration in frozen cauliflower; and further studies on the use of antibiotics in food preservation.

Frozen Concentrated Apple Juice

During the years 1950 and 1951, a special committee appointed by the Washington State Horticultural Association has collaborated with various research agencies in a full-scale study of commercial feasibility of frozen concentrated apple juice. The objective is an economical outlet for apples of good juice quality but lower grade as far as fresh fruit marketing is concerned.

This cooperative enterprise has involved process development and consumer-preference and marketing tests. The collaborating agencies are the Western Regional Research Laboratory of the Bureau of Agricultural and Industrial Chemistry, the Bureau of Agricultural

Economics and the Washington Agricultural Experiment Station. The special committee of the apple industry is called the Industry Apple Concentrate Committee.

The first step was to determine the varietal blend to be used in preparation of the market test samples of frozen apple juice concentrate. The possibilities included various combinations of Standard Delicious, Winesap, Jonathan, Rome Beauty, and Golden Delicious varieties. All blends tested contained at least 50 percent Delicious apple juice, as this roughly represents the proportion of this variety in Washington. Discrimination and preference tests, carried out by the Washington Experiment Station, narrowed down possibilities to three, representing the top quality of frozen apple juice concentrate obtainable from the raw material. These three categories (straight Delicious, acidified Delicious, and a blend of Delicious, Winesap, Jonathan, and Rome Beauty mixed in the ratio 5:2:2:1) were then submitted to a consumer preference test, carried out by the Bureau of Agricultural Economics in the San Francisco Bay area. The test showed definite preference for the blend and the acidified Delicious.

Upon completion of the consumer preference test, about 40,000 six-ounce cans of 4-fold frozen apple juice concentrate were prepared by the Western Regional Research Laboratory. The method of preparation for the concentrate was as follows: The volatile flavor was stripped from fresh juice and the stripped juice was concentrated to slightly over 4-fold. The essence was returned to produce a 4-fold product, which was immediately frozen, stored, and handled as frozen product. The process of stripping and concentration of the essence has been described by the Eastern Regional Laboratory. A modification of this process, involving a steam-injection heater developed by the Western Regional Laboratory, was used for these studies. Concentration was effected in a vacuum pan. The Western Laboratory has shown that this step can be carried out without impairment of quality at temperatures as high as 130°F. Thus, less expensive equipment can be used for concentration of apple juice than for orange juice, where evaporative temperatures below 85°F. are ordinarily used. The market test on the frozen apple juice concentrate has been conducted cooperatively by the Washington State Apple Commission and the Bureau of Agricultural Economics. Results of the whole project are being evaluated and a report will be made as a basis for recommendations on future industrial action.

New Uses for Fruit Cannery Wastes

The project on utilization of all waste of a fruit-processing plant for the manufacture of feeds (molasses and dry pomace) has reached the final stages of pilot-plant study. The past year's work has produced improvements in pretreating and dejuicing of waste and good results from feeding tests with the products. Continuous, low-scale operation of the plant at San Jose during the present year will provide a basis for final evaluation of full-scale operation. The Western Regional Research Laboratory conducts this project cooperatively with the Canners League of California.

The project on use of clean pear cannery waste for the production of sirup suitable for use in canned product has reached a stage of readiness for commercial adoption. The amount of canning sirup provided by the process and the quality of fruit canned with the sirup have been sufficiently satisfactory to justify commercial application of the method. In this work a cooperative association of fruit growers in Oregon has collaborated with the Bureau's Fruit and Vegetable Products Laboratory (formerly in Pullman, now located in Prosser, Washington).

Feed and Molasses from Cannery Wastes:

Pilot plant studies were continued during the 1950 season, in cooperation with the Canners League of California, who supplied plant facilities and financial assistance. The Cling Peach Advisory Board also assisted with financing. Operations were improved, as a result of progress made during the 1949 pilot-plant studies at San Jose, and also as a result of process studies conducted on a laboratory scale during the winter months of 1949-50. The laboratory studies led to two important developments: a greatly improved process for treating pear waste to facilitate separation of juice from pomace and a new type of dejuicing machine for effecting the separation of juice from pomace. Both developments were applied successfully on a pilot-plant scale during the period of August 14 to October 16, 1950.

The new treating process proved to be relatively simple in use and highly flexible. Liming of ground waste under controlled conditions, in a two-stage process, converts it from a slimy mass to a form suitable for separation into juice and pomace. The process works satisfactorily on wastes from pears of a full range of varieties and maturities, and on mixed wastes containing pear, peach, grape, and tomato. The new dejuicing machine greatly facilitates processing by combining dejuicing of waste and pressing of cake in a single, continuous, easily controlled operation. Previously used commercial equipment required separate machines for dejuicing and pressing pomace.

Approximately 140 tons of pear waste were processed during the season. The pear molasses and dry pear pomace were used in stock feeding and other product utilization tests. Feeding tests made by the California Agricultural Experiment Station showed that pear molasses, or pear pomace containing 20 percent pear molasses, is very palatable to sheep and cattle and should find a ready market as feedstuffs. Pear pomace proved acceptable as a carrier for insect baits.

During the past winter, a continuous treater was developed for converting ground pear waste from its usual slimy condition to a granular form which readily releases its juice in the continuous press. The development promises to be another major contribution to the field of fruit waste utilization. The Canners League has

appropriated $65,000 for further pilot studies during 1951 to evaluate the new continuous pear waste treater and an improved model of dejuicing machine. The research phase of the project should be brought to a satisfactory conclusion during the 1951 season.

Sugar Recovery from Pear Canning Waste:

A process for the recovery of sugar from pear canning waste was operated on a semicontinuous pilot-plant scale during the 1950 pear canning season by the Bureau's Fruit and Vegetable Products Laboratory, Pullman, Washington (now located in Prosser, Wash.) The process recovers the sugars and other soluble solids from peeling and core waste. The sugars are recovered as a clear, colorless juice that the canner may use as a sirup base in canning pears. Sugars recovered by the process would replace about one-third of the refined sugar normally used in canning this fruit.

The pilot-plant operation was conducted in cooperation with the Apple Growers Association, a farm cooperative located in Hood River, Oregon, in the Association's cannery. The technical practicability of the process was demonstrated and operational details were established. During the study 2500 gallons of juice were refined and used in preparing 1500 cases of canned pears for use in consumer acceptance tests by the Association. Pears canned in juice sirup are considered equal in appearance and in flavor to similar fruit canned in the usual sugar sirup.

Discoloration of Cut Surfaces of Fruits

Recent investigations at the Western Regional Research Laboratory have contributed in several respects to an understanding of the problem of browning on cut surfaces of fresh and thawed fruits. In addition to detracting from the appearance, the browning also reduces nutritive value because it is accompanied by loss of vitamin C.

A histochemical test has been developed for determining the distribution of the tannins within the fruit tissues. This test indicates the portions of fruit most susceptible to browning.

In experiments on isolation and identification of the tannins in peaches, modern techniques such as chromatography and countercurrent extraction have identified caffeic acid as an important browning substrate. This information is useful in studies on browning and may prove valuable to plant breeders in the development of a chemical method for rapid testing of the browning susceptibility of new strains of fruits in the seedling stage.

Since chlorogenic acid has been reported as a probable browning substrate in fruits, the rate of its non-enzymatic oxidation by oxygen has been investigated. The initial rate was found to be directly proportional to the concentration of chlorogenic acid and

pressure of oxygen, and inversely proportional to the concentration of hydrogen ion. The rate law has been interpreted in terms of a mechanism which postulates the formation of a semiquinone as an intermediate. The results will make it possible to interpret the contribution of non-enzymatic oxidation in later studies of the enzymatic oxidation of the tannins.

A study has also been made of the role of ascorbic acid in the enzymatic oxidation of catechol. Ascorbic acid reacts with oxidation products of the catechol and thus inhibits the browning until all the ascorbic acid has reacted. During this process some of the enzyme also is destroyed. When an excess of ascorbic acid is used, the amount eventually oxidized is a function of the initial amount of the enzyme present. Studies with apple enzyme have shown a first-order rate dependence and have been interpreted in terms of the destruction of enzyme by a semiquinone free radical. These results may lead to the development of a much simpler method of measuring polyphenolase activity than has previously been used.

Heat Required to Control Browning in Purees of Various Fruits

For the fruit puree industries and for further research on fruits, precise knowledge of the heat required to inactivate the enzyme that causes browning is valuable. Even slight underheating will result in discoloration, and overheating reduces fresh flavor. To supply more accurate information than has been available, the Western Regional Research Laboratory has investigated the destruction of polyphenolase in several fruit purees at various temperatures and under various conditions of pH and maturity of the fruit.

From carefully made rate measurements on purees of grape, peach, apricot, apple, and pear it has been possible to estimate the time and temperature conditions for essentially complete inactivation of polyphenolase. It was found that different conditions are required for the different fruits. For a given fruit, the required heating time increases very markedly for a small decrease in temperature. It was also found that the inactivation time is greatly affected by changes in pH and that each fruit can be characterized by a pH of maximum enzyme stability.

These results, which were obtained with certain selected samples of fruits, will serve as valuable guides for further research. It is also evident that fruits of different variety, maturity, or from different localities, may require somewhat different heat treatments because of variations in composition. The work is being continued to determine the effect of these variables on the inactivation requirements for several commercially important fruits.

Analysis of Volatile Constituents of Strawberries

Information on identification of the volatile components of fruits has important applications in fruit processing and the manufacture of commercial essences. A method of measuring essence strength, for example, is needed, and likewise a method for measuring the amounts of ethyl alcohol that essence preparations may contain. Fruit breeders, pomologists, and others may find use for such information.

Investigations at the Western Regional Research Laboratory on the preparation of strawberry essence and analysis of its constituents have contributed much of the desired information. These studies were made in cooperation with the Western Washington Experiment Station, at Puyallup, on several varieties of strawberries grown in the Pacific Northwest.

A quantitative method was developed for estimation of total amount of the volatile organic material removed from the fruit by distillation. With this method it was found that approximately 50 percent of the water must be flash-vaporized from a strawberry puree to recover about 90 percent of the flavor. It was shown further that excessive heating of the aqueous distillate causes flavor degradation, which precludes concentration of the flavor by batch distillation. Distillation in a continuous still is, however, satisfactory. It was found also that a homogeneous strawberry concentrate cannot be prepared in strength greater than about 300-fold, because of the formation of two immiscible liquid layers.

Flavor evaluation of seven different varieties of Northwest strawberries showed that they vary markedly in flavor intensity. That this variation is probably characteristic of the varieties is indicated by the observation that approximately the same order of intensity for the different varieties was found for two different harvest seasons.

Chemical identification of the volatile organic components was carried out on the essence of Marshall strawberries, the most important commercial variety in the Northwest. About 90 percent of the organic material was found to consist of biacetyl, acetaldehyde, acetone, 2-hexenal, methyl alcohol, ethyl alcohol, several acids and some unidentified esters. About 50 percent of the non-aqueous material was found to be ethyl alcohol.

A strongly aromatic fraction, comprising about 10 percent of the organic material, still remains to be identified. The work is being continued and will be expanded to include a study on preparation of high-strength concentrates and on determination of the factors that govern their stability, especially in highly concentrated fruit products such as are needed in connection with the defense effort.

Dehydration of Vegetables

During World War II dehydrated vegetables served a highly useful purpose, despite the fact that military experience, especially with regard to palatability and convenience, was not entirely satisfactory. Nearly all of the war-built vegetable dehydration industry discontinued operations at the close of the war. Those companies that continued were largely those that had dehydrated vegetables before the war. The Western Regional Research Laboratory discontinued most of its research on dried vegetables with the exception of a few basic projects aimed at an understanding of fundamental causes of difficulties.

The present rearmament program has now imposed a new time table for the solution of outstanding problems in vegetable dehydration, and the Western Regional Laboratory has established close liaison with the Quartermaster Corps and has undertaken several investigations of immediate concern. A comprehensive survey of published information about a relatively new product, potato granules or dehydrated mashed potato, has been issued in mimeographed form. The storage stability of the commercial product has been investigated, and laboratory studies of the characteristic processing steps undertaken, with a view to discovery of the causes of certain troublesome quality defects. Other work suggested by the Quartermaster Corps has included studies of the techniques of sulfiting dehydrated vegetables, development of acceptable dehydrated green peas and string beans, solution of several problems in the dehydration of sweetpotatoes, and development of better analytical methods for production control and inspection.

During World War II the Bureau of Agricultural and Industrial Chemistry sponsored the publication of a Department bulletin summarizing dehydration technology for the benefit of plant operating personnel. This bulletin, now out of print, is being completely revised; two of the rewritten chapters, on the principles of drying and the characteristics of tunnel dehydrators, have been issued as mimeographed separates. At the request of the Quartermaster Corps, and financed by a grant from the Corps, a "preparedness study" of dehydration is being compiled as a handbook which can be used by the Corps to inform industrial management if it becomes necdssary again to expand the dehydration industry rapidly.

Keeping Quality of Dehydrated Vegetables

Recent expansion of military preparations has created a need for improvements in dehydrated vegetables. Civilian markets for certain products--notably "potato granules" (mashed potato powder)--also have expanded. Several deteriorative processes occur during storage, however, and limit the usefulness of dehydrated vegetables. These changes include the development of off-flavors and odors, degradation of color, impairment of rehydration characteristics, and destruction of vitamins. Comprehensive studies at the Western Regional Research Laboratory have provided quantitative information

on a number of factors affecting the storage stability of dehydrated white potato, sweetpotato, cabbage, carrot, beet, and onion.

The most important type of deterioration is browning, which is due to the so-called "browning reaction". This reaction was particularly troublesome during World War II. It occurs between protein and other nitrogenous constituents and certain sugars and, unlike enzymatic discoloration, is not prevented by heat treatment. Because it proceeds much more rapidly at elevated temperatures, dehydrated vegetables deteriorate very quickly under tropical conditions. The reaction results not only in brown color but in an unpleasant scorched flavor and in difficulty with rehydration.

Quantitative measurements have shown that substantial protection against this reaction is obtained by treatment with sulfite solutions and by drying to a low moisture level. Protection adequate even for military purposes appears obtainable by combining these two treatments. In a typical lot of dried non-sulfited white potatoes, detectable browning developed in 9 days at 100°F. Comparable sulfited samples at 4 percent lower moisture content withstood 160 days' storage at that temperature before detectable browning occurred.

Low moisture has been found especially beneficial for dehydrated cabbage and onion. For these commodities, which are particularly susceptible to browning, the storage life at 100°F. was increased 8- to 10-fold by "in-package desiccation" (packing a drying agent in the final container with the dehydrated vegetable). Very low moisture levels can be conveniently obtained by this process.

The rate of browning was found to double with an increase in temperature of only 6° or 7°F. Browning is thus remarkably sensitive to temperature, since the rates of most common chemical reactions double with a 12 to 18 degree temperature rise. It was also established that in the low moisture range the rate is approximately doubled by an increase in moisture content of 2 percent.

Packing dehydrated vegetables in vacuum or an inert gas such as nitrogen or carbon dioxide was shown to retard destruction of ascorbic acid (vitamin C) and carotene (provitamin A) during storage. It also greatly retards the development of rancid odors and flavors, but has little if any effect on browning. Loss of vitamin C was also markedly retarded by lowering the moisture content.

These studies have demonstrated that dehydrated vegetables of greatly improved storage stability can be prepared by a combination of sulfiting, drying to low moisture content by in-package desiccation or otherwise, and inert gas packing. Such high-stability products will be of special value for military use in areas where high temperatures prevail in storage dumps and warehouses.

Rapid Determination of Sulfite in Dehydrated White Potato

Specifications for military purchase of dried white potato require 200 to 500 parts per million of sulfur dioxide in the product to protect it against deterioration from exposure to high temperature. Since sulfite is applied by spraying or dipping prior to dehydration and since the product absorbs sulfur dioxide from fuel oil combustion gases in direct-fired dehydrators, plant operators require an analytical method to control sulfur dioxide content within the specified range.

The distillation method described in present Quartermaster specifications is time-consuming and not well adapted to processing plants where a minimum of equipment is desired. A rapid method (developed at the Western Regional Research Laboratory) is now coming into use. It compares very favorably in precision and accuracy with the distillation method. Both elapsed and working time have been cut sufficiently so that an operator can make about four times as many analyses per day. The technique is simple and therefore suitable for use by non-technical operators in dehydration plants and for testing for compliance with specifications. Standard laboratory equipment and common reagents easily obtainable are used. The method of sample preparation commonly used is satisfactory.

For analysis a representative portion of dehydrated potato is ground and two samples are weighed into beakers. The samples are treated with a solution of sodium hydroxide. The mixture is stirred gently, to avoid incorporation of air in the solution, and allowed to stand for 20 minutes. One sample is acidified with hydrochloric acid, starch solution is added, and the sample is titrated immediately with a standardized iodine solution to a definite dark blue color. The titrations are best made while a mechanical stirrer, carrying a propeller with broad blades having a steep pitch, incorporates the iodine into all parts of the solution without beating air into it. This titration measures the sulfite content together with the nonsulfite reducing materials. To determine the reducing material other than sulfite, the second sample is acidified and formaldehyde solution added to bind the sulfite. With the mechanical stirrer in operation, the solution is rapidly titrated, preferably with the stopcock fully open until near the end point, and then with small increments added in quick succession until a dark blue color persists for at least 15 seconds. The difference between the two titrations is a measure of the sulfite content of the dehydrated white potato.

Pink Centers in Frozen Brussels Sprouts Due to Inadequate Scalding

Frozen Brussels sprouts occasionally develop pink centers, a defect commonly associated with loss of flavor, off flavor, and loss of grade. Recent investigations at the Western Regional Research Laboratory have shown that scalding to the extent necessary to inactivate peroxidase prevents this difficulty.

The effect of degree of scalding on quality of frozen material received first consideration, from the standpoint of over-all flavor as well as pink centers. Samples prepared from the same lot of raw material were scalded in steam 2, 3, 4, 5, 6, and 10 minutes. These samples, after evaporation-fog cooling, were packaged, frozen, and stored at -10°F. for 10 months. Enzyme assays were carried out at the time of processing and at the end of storage period by methods developed here previously. Peroxidase was determined by both a semi-quantitative test developed for processing plants and by a quantitative test; guaiacol and hydrogen peroxide were used as substrates in both tests. Catalase was determined by a quantitative test in which rate of disappearance of hydrogen peroxide was used as a measure of catalase activity.

At the end of the storage period, taste panel evaluations showed that all samples scalded to a point sufficient to inactivate peroxidase maintained quality over the ten-month storage period at -10°F. On the other hand, samples which had appreciable amounts of residual peroxidase had a marked off-flavor, decreased natural flavor, and pink color at the centers. These data indicated clearly that lowering of quality and the development of pink centers can be prevented by scalding to a degree sufficient to inactivate peroxidase as determined by the assay methods used. Catalase, because of its greater heat lability and because of its apparent inactivation during freezing storage, was found to be unsatisfactory for this purpose.

Subsequent investigation has shown that pink centers develop immediately after scalding. In under-blanched material, the rate and degree of discoloration can be greatly enhanced, for laboratory experimental purposes, by addition of a dilute solution of hydrogen peroxide to the surface of the cut halves.

It was found that scalding to complete peroxidase inactivation is not necessary for prevention of pink center development, since samples which had only about 10 percent of residual peroxidase retained flavor and failed to develop pink centers after twelve months of storage at -10°F. In spite of this fact, scalding to complete inactivation of peroxidase is advocated in order to insure a certain margin of safety.

Scalding Sweet Corn on the Cob Improves Quality

It has seemed probable that scalding sweet corn on the cob to inactivate enzymes in preparation for freezing would have advantages over scalding of cut kernels. That is, if the hot steam hardens the protein and starchy materials, there should be less possibility of loss of these materials and also the sugars during scalding and during subsequent fluming in water. On the other hand, scalding kernels rather than whole ears puts less demand on the scalding equipment.

Since experimental data regarding the comparative advantages of cob-blanching and blanching of cut kernels were meager, studies have been carried out by the Western Regional Research Laboratory in cooperation with the Utah State Experiment Station and the Irrigation Experiment Station of Washington. The Golden Cross Bantam variety was used. Harvests were made at four levels of maturity, with moisture contents ranging from 63 to 77 percent. At each harvest, samples were prepared by six procedures.

Chemical analyses and taste testing showed highly significant differences between the frozen samples prepared by the two methods, and the advantage was in favor of material scalded prior to cutting. Samples scalded before cutting, although flumed after cutting, were higher in total solids, total sugars, and weight per kernel than samples cut and flumed prior to blanching and then flumed afain after blanching (a process used in many commercial operations).

The cob-blanching procedure apparently alters the kernel contents by gelation of the starches and proteins, so that loss of soluble materials is minimized. As a result, loss of nutrient material is minimized and the overall yield of frozen product is significantly increased.

Thus the results of chemical analyses and taste tests have demonstrated a distinct advantage of blanching corn on the cob prior to cutting. By this procedure, which is easily adaptable to commercial operation, a frozen product of higher quality is obtained in greater yield.

Measuring Maturity of Sweet Corn

The maturity of sweet corn at harvest determines to a large extent the quality of canned or frozen product. Ability to harvest at optimum maturity and to segregate the several maturities found in harvested corn is essential. Processors are therefore in need of an objective measure of maturity that is sufficiently accurate, rapid, and simple to permit its use in production and quality control. Starch and moisture content have been established as accurate indexes of maturity, but usual methods for their determination are not sufficiently rapid for plant operations and the thumb-nail test is still widely used to determine picking dates.

The relative merits of several methods have been evaluated by the Fruit and Vegetable Products Laboratory in Pullman, Washington (now located in Prosser). In a three-season study, it was determined that measurement of refractive index of juice expressed from a representative sample met the commercial processor's requirements of simplicity, reliability, and speed.

Studies were conducted in cooperation with the University of Idaho to determine factors affecting the distribution of maturity within a single field lot of harvested sweet corn. The results showed considerable variation in relative proportions of young, prime, and mature corn in a single harvest. This variation is related to both season and

field-run moisture content. There is also a considerable increase in total yield of cut corn as it becomes more mature. Consideration of probable total yield and proportions of each maturity grade expected at different moisture levels enables processors to regulate harvest schedules more closely and obtain the greatest possible yield of optimum maturity.

Measurement of Moisture Content of Fruits and Vegetables by New Methods

Vacuum-oven methods of measuring moisture in processed fruits and vegetables are used by the armed forces in the procurement of foods but these methods require too much time for efficient use in commercial processing plants that produce the foods. A new approach has been made at the Western Regional Laboratory in the development of a simple, rapid, reproducible procedure in which the non-aqueous content of a specimen is measured by the quantity of potassium dichromate required to oxidize it. The water content of the specimen is determined by difference. The method is particularly suitable for materials of high moisture content such as fresh fruits and vegetables, but may also prove useful for dehydrated products.

The procedure is simple. A measured volume of dichromate solution is added to the suitably prepared sample, concentrated sulfuric acid is added, and the heat of dilution provides instantaneous reproducible heating. Excess dichromate is then titrated electrometrically with ferrous ammonium sulfate solution. An inexpensive circuit of a microammeter, dry cells, and radio potentiometer can be used for this titration, or either a pH meter or commercial titrimeter may be used. The sample need be only coarsely ground or slurried. From 5 to 12 minutes are required for a single determination exclusive of the time required for sample preparation. For any sample approximately 12 determinations can be made per hour. The dichromate used is converted to weight of non-aqueous material by use of a factor determined for each material by use of a reference method for determining moisture. The difference in weight of specimen and of non-aqueous material is then the moisture content on the scale established by the reference method.

Although the factor for a given material may be expected to show variations with variety, maturity, and cultural conditions, use of an average value has been found to result in only moderate errors in moisture content. Thus mean errors of less than 0.5 percent in moisture content were found with a wide variety of samples of dehydrated potatoes, dried prunes, and fresh peas by use of an average factor for each substance based on vacuum oven reference procedures. Reproducibility, a consideration even more important than accuracy for some applications, is better than 0.2 percent for each of these materials. The factors for such different materials as potatoes, prunes, peas, carrots, and a pudding consisting of rice, pineapple, milk, sugar, and eggs, are the same within 6 percent.

Chemical Composition of Pectin

Recent investigations at the Western Regional Research Laboratory have resulted in identification of the components of the so-called non-uronide portion of pectic substance and have shown that this non-uronide material consists of three sugars which appear to be an integral part of the pectin molecule. This finding has a direct bearing on problems such as rehydration of dehydrated fruit, extraction of pectin for commercial uses, and formation of pectin jellies, jams, and marmalades.

Pectin is the carbohydrate familiar as a jellying agent in foods. It occurs in nearly all higher plants, being particularly prominent in peel of citrus fruit, apple pomace, and sugar beets. Because of its properties and location in the intercellular tissue, it plays an important role in control of texture of many fruits and vegetables. The amount available for commercial production and use is considerable--nearly 90,000 tons in peel from citrus processing alone.

The scientist knows pectin as a polymer of galacturonic acid, a simple sugar in which the end group is an acid instead of the usual alcohol. About 75 percent of the acid groups in pectin are esterified with methyl alcohol. In addition, 10 to 40 percent of pectic substances is neither uronic acid nor its methyl ester. Scientists have long been puzzled by this non-uronide and have wondered whether it is part of the pectin molecule or merely associated as ballast material. At the Western Regional Research Laboratory, application of chromatography, X-rays, and crystallography, has shown the non-uronide to be composed of arabinose, galactose, and rhamnose. The last-named sugar had not been previously identified in pectin preparations.

In agreement with results obtained at other laboratories, it was found that precipitation of pectin from water solution with alcohol or copper salts did not remove the non-uronide. Even deesterification of the pectin followed by extraction of the resulting pectic acid with boiling water did not remove the contaminant. Extraction of pectin with hot 70 percent methyl alcohol removed some of the non-uronide but not in sufficient quantity to be conclusive. Application of an electric current to pectin solutions caused the pectin to move to the anode and this pectin had lost some araban but most of the non-uronide went along even though it possessed no charged group. All this evidence favors the hypothesis that non-uronide is part of the molecule.

If the pectin is deesterified by alkali, precipitated several times by acid, and then subjected to an electric current, a fairly pure polygalacturonide concentrates in the anode chamber. Also, if pectin is esterified with propionic anhydride and then deesterified with alkali, most, if not all, of the non-uronide is removed. These results lead to the hypothesis that the non-uronide is held to pectin either by ester groups as part of the molecule or held by hydrogen bonds.

The Physiological Action of Rutin and Related Flavonoids

The flavonoids are rather widely distributed in plants used for food, as exemplified by the rutin in asparagus, the quercetin galactoside in the skins of certain varieties of apples, the hesperidin in citrus fruits, and a phlobotannin-like substance in grapes. Investigations of the Pharmacology Laboratory have shown that a number of these flavonoids possess the properties responsible for so-called "vitamin P" activity, and that rutin has a sparing action toward vitamin C and adrenalin. This sparing action appears to be due to the antioxidant properties of rutin, which retard the oxidative destruction of vitamin C and adrenalin, thus permitting small amounts of these two compounds to accomplish the physiological results of larger amounts.

The sparing action toward vitamin C was demonstrated by the fact that scorbutic guinea pigs receiving rutin together with subminimal amounts of vitamin C survived significantly longer and showed fewer fresh hemorrhages than animals given the same amount of vitamin C alone. Investigations at McGill University have confirmed the work of the Pharmacology Laboratory. They showed that administration of rutin permitted a 50 percent increase in relative potency of known amounts of vitamin C given to scorbutic guinea pigs as judged by developmental changes of the incisor teeth.

These observations are of practical importance for several reasons. Attention was focused first on the possible nutritional physiological importance of the flavonoids in 1936 by the report that a beneficial effect on vascular permeability could be demonstrated experimentally in guinea pigs. Perhaps the most frequently stated physiological effect of rutin concerns its alleged ability to restore capillary fragility and permeability to normal. However, rigorous objective proof that rutin modifies the porosity or fragility of capillaries is lacking. In fact, it may be doubted that increased permeability and fragility of the capillaries exist in scurvy, a disease in which such conditions of the capillaries have been supposed to be characteristic. Recent studies by two qualified groups of investigators have shown that the capillaries of scorbutic guinea pigs do not exhibit increased permeability. The demonstrated sparing action of rutin toward vitamin C and adrenalin affords an alternate and satisfactory explanation. The responsiveness to adrenalin of the smooth muscular structures controlling blood flow in the capillaries tends to be maintained and the decreased oxidative destruction of circulating adrenalin permits a more persistent constrictor action of adrenalin on these muscular structures. The net result is a decrease in tendency to capillary bleeding.

The concept of antioxidant activity as a basis for mechanism of rutin action provides a more rational basis for the therapeutic use of rutin in conditions where abnormalities of the capillaries need not be visualized, as in hemophilia, idiopathic methemoglobinemia, and arthritis where a supplemental action to vitamin C may

be desirable. Finally, the antioxidant actions of rutin and related compounds together with their known effects on various enzymes suggest the importance of the flavonoids as a dietary factor which helps to regulate physiological processes.

New Method of Counting Spores Aids Research on Clostridium Botulinum

Commercial food canning procedures must include sterilization to an extent adequate to destroy Clostridium botulinum, because this bacterium is capable of producing a deadly toxin. Consequently research workers use this organism and also the similar but somewhat more heat-resistant putrefactive anaerobe 3679 in various kinds of research and testing. Such work has been hampered by an inadequate method for the counting of spores of C. botulinum in samples. Recent research at the Western Regional Research Laboratory has developed a method that makes accurate counting possible without long delay.

To count the number of viable spores in a sample, it is necessary that they germinate and form colonies, but C. botulinum exhibits "dormancy" to a marked degree; that is, germination of many of the spores is delayed in ordinary media. Thus workers have often obtained low spore counts. Several bacteriologists have observed that carbon dioxide is beneficial to the growth of C. botulinum, but apparently this fact has not been used to improve counting methods.

The Western Regional Laboratory has now developed a procedure which makes possible accurate spore counts with only 24 to 40 hours of incubation time. It is based on the use of a nutrient medium containing sodium bicarbonate as a source of carbon dioxide and of a glass plate imbedded in agar in a Petri dish. The method has been tested with 8 spore preparations of C. botulinum, Types A and B, and with Putrefactive Anaerobe 3679. In each case the inclusion of bicarbonate improved rapidity and completeness of germination. In 4 cases where the colony count had reached essentially a maximum in 24 hours in the presence of bicarbonate, only 2 to 12 percent of the spores had formed visible colonies in its absence. Three spore suspensions calibrated at other laboratories and by other methods were counted by the new procedure and were found to contain from 2 to 4.5 times as many viable spores as had been reported. At least one large commercial research laboratory is now using this counting method, not only for C. botulinum and Putrefactive Anaerobe 3679 but also for thermophilic and butyric acid anaerobes.

New Basic Analytical Methods

In studies on isolation and identification of flavoring constituents of citrus fruits, it has been necessary to find a method to isolate and purify the small amounts of terpene flavoring material present. This has been accomplished by a new technique in

chromatography developed at the Bureau's Fruit and Vegetable Chemistry Laboratory in Pasadena, California, which is useful not only in terpene chemistry but also in organic chemistry as a whole.

The technique consists in coating glass strips (1/2 x 5 inches) with an adsorbent mixed with either starch or Plaster of Paris as a binder. The substance to be chromatographed is spotted near one end of the strip and then developed with the aid of capillary attraction by dipping the end of the strip in a suitable solvent. The solvent is then evaporated and the various chromatographic zones of the colorless compounds are indicated on the strip by spraying with a suitable reagent. Compounds which adsorb bromine can be located by spraying with fluorescein solution and exposure to bromine vapors. Those which adsorb bromine faster than fluorescein, such as those containing ethylenic-type double bonds, show up as yellow zones on a pink background. Very unreactive compounds can be located by spraying the strips with a concentrated sulfuric-nitric acid mixture and heating to cause charring of the compounds. This particular test is useful in making sure that no compound, which is indifferent to the usual tests for finding colorless compounds on chromatographic columns, eludes the investigator.

This "chromatostrip" technique is a micro method and provides a rapid means of checking solvents and adsorbents for use on larger columns. Rf values can be used to assist in the identification of compounds. To isolate sufficient material for chemical analyses, the method was extended to a larger column, which is self-supporting and not encumbered by a glass envelope. It is formed by mixing an adsorbent with Plaster of Paris and casting in the form of a bar. A special solvent distributor has been designed to distribute solvent uniformly to the bar, and development is accomplished in a glass cylinder. The bar can be removed for observations of chromatographic development, and can then be returned to the same solvent or a different one for further development. Here is an advantage over the usual packed chromatographic column, because once the latter is removed from its glass envelope, no further development can be undertaken.

Identification of Nitrogenous Components of Citrus Juices

Darkening and development of off-flavors are deteriorative processes that require control during the processing and storage of citrus juice products. Citrus-fruit research workers have suspected for many years that unknown nitrogenous compounds in citrus juices may contribute to the unfavorable reactions that result in loss of characteristic properties of fresh juices. In addition, it is well known that amino acids and other organic-nitrogen compounds react with sugars, such as are known to be present in citrus juices, with the formation of unusual colors, flavors, and odors. Nitrogenous constituents make up almost 10 percent of the solids in citrus juices.

During the past few years scientists have developed a number of new methods for the determination of amino acids and other nitrogenous substances. One of the most rapid and convenient, called partition chromatography, is applicable to qualitative and quantitative estimation of many types of compounds present in complex mixtures. The Fruit and Vegetable Chemistry Laboratory at Pasadena, California, has recently developed a series of rapid, small-scale filter-paper-partition chromatography techniques which can be applied to fruit and vegetable juices and extracts. These have been applied to the qualitative analysis of nitrogenous compounds in fresh and processed Valencia and Navel oranges, grapefruit, lemon, tangerine and lime juices extracted from California, Florida, and Texas fruits.

It was found that organic nitrogen in citrus juices is present largely in the form of free amino acids and other simple molecules. The various citrus juices contained the nutritionally important amino acids (alanine, aspartic acid, asparagine, glutamic acid and serine) in amounts approximating the vitamin C content of the juices. In addition, Valencia orange juice contained arginine, proline and another major constituent believed to be sarcosine, while proline but not arginine nor sarcosine were found in Navel orange juice. Grapefruit and tangerine juices were identical in respect to their major nitrogenous constituents, containing arginine in addition to the 5 amino acids present in all the juices, while lemon juice contained only the latter compounds. Among the important minor nitrogenous constituents identified are cysteine and glutathione. The Enzyme Research Division of the Western Regional Research Laboratory previously reported the identification of these compounds in orange juice. Quantitative filter-paper partition chromatography of fresh and heat-treated orange, lemon, grapefruit and lime juices have shown that all of these juices contain small but significant amounts of both cysteine and glutathione, and that approximately 25 percent of each of these compounds is destroyed by heating. The foreign flavors and aroma developed in processed citrus products may be due in part to the formation, from these two sulfur-containing compounds, of hydrogen sulfide and other sulfur-containing decomposition products.

The presence of relatively large amounts of free amino acids in citrus juices indicates that the role of these compounds in deteriorative changes of processed fruit juices may be even more important than previously suspected. This is particularly true in concentrated juices, where more nearly optimum conditions are provided dor the Maillard ("browning") reaction. Further work must be done to determine the relationship between individual nitrogenous constituents and the deterioration of citrus juice products.

Frozen Avocado Puree or Guacamole

Although the avocado is not produced in oversupply at present, yearly increases in plantings in Southern California are being made and surpluses may occur in the near future. Studies recently carried out at the Fruit and Vegetable Chemistry Laboratory in Pasadena, California, have resulted in a new processed product that may add to the variety of the American diet and also serve as an outlet for surplus and cull fruit.

Attempts to preserve avocado slices and halves have never succeeded because of the extreme rapidity with which tissues of the fruit turn brown on exposure to air or during heating. Products such as sandwich spread and salad dressing mixtures, as well as slices and halves preserved by pickling, have been marketed in small quantities.

A popular avocado spread called "guacamole" is made from pureed avocado, salt, lemon or lime juice, and onion or garlic. This spread retains to a high degree the desirable, unique flavor of the fruit, but it turns brown in four to eight hours after preparation, even when refrigerated. Experiments were conducted to improve the color retention of this product by varying the proportions of ingredients used in the recipe. As a result, a formula was developed for a paste which retains attractive green color without darkening for at least a year in frozen storage, and for one to two weeks after thawing and storage at refrigerator temperatures. This has been accomplished primarily by adding larger proportions of lemon or lime juice and salt to the puree. Avocado paste can be manufactured from overripe or second-grade fruit, and when prepared and properly handled, a desirable product, suitable for distribution as a frozen food, is obtained.

The method is comparatively simple. Sound, ripe fruit is thoroughly washed, preferably with a good detergent, and rinsed well with cold water to reduce microbial contamination. The fruit is pared and all discolored spots, damaged portions, and the seed are removed. The flesh is then pureed by sieving or grinding. The avocado puree is then mixed with lemon or lime juice, salt, and onion powder in the following proportions (by weight): Avocato puree, 100 parts; lemon or lime juice, 8 to 10 parts; salt (sodium chloride), 1 to 2 parts; and dehydrated onion powder, 0.3 part. The ingredients are blended and the finished product filled into a suitable container and frozen at 0° to -10°F. The quantity of onion powder can be varied according to taste or omitted. Garlic powder can be used to vary flavor.

Glass, enamel-lined tin, plastic, and wax-impregnated-fiber containers are suitable. However, the most novel and convenient container is a collapsible aluminum or tin tube. With this container, the withdrawal of small portions of thawed product as consumed leaves no headspace for contact with air which might accelerate discoloration. Avocado paste prepared and packaged as described has retained satisfactory color and flavor for one year at 0°F. to -10°F. and for one to two weeks at refrigerator temperature after storage.

POULTRY, DAIRY, AND ANIMAL PRODUCTS UTILIZATION INVESTIGATIONS

Further work has been done on problems relative to the storage stability of dehydrated eggs, and a careful comparison under commercial conditions is being made on the merits of two processes (acidification and glucose removal) that have both been found to be effective. Other work under way includes: development of chemical tests for rancidity and characterization of fat in poultry; studies of the mechanisms of rancidity; and poultry dressing (scalding time, temperature, etc.) technology. Fundamental investigations on egg white proteins including their isolation, characterization and determination of antibacterial activity were concluded during the year. A more complete report on some additional projects is given below:

Causes of Deterioration in Eggs

Although commercial handling preserves fresh quality of shell eggs with considerable success, it may be possible to make further and more economical improvements through advancement of knowledge of the chemical causes of the deteriorative processes that are known to occur. The chief processes of change are thinning of the white, weakening and flattening of yolks, and loss of moisture. One approach is a study of effects of specific chemical reagents on eggs and a comparison of changes caused by chemicals with deteriorations that occur during storage.

Recent studies at the Western Regional Research Laboratory, continuing earlier studies of this Bureau on chemistry of eggs, have demonstrated striking results by this approach. Changes simulating natural deteriorations have resulted from addition of minute amounts of any one of several chemical reducing agents. Depending upon amount added, manner of administration, and time of action, varying degrees of thinning of the white and weakening of the yolk resulted, which were similar to changes found under ordinary conditions. Among reducing chemicals that caused these changes were hydrogen sulfide, sulfur dioxide, thioglycol, thioglycollic acid, cysteine, and cyanide.

The action of the reducing chemical was shown to take place on the membrane of the yolk and on the ovomucin fraction of egg white (the ovomucin fraction is considered to be responsible for the gel-like structure of thick egg white). The nature of the action was probably the same as occurs with certain other fibrous-type proteins. Disulfide bonds in the structure of these proteins are broken when the materials are treated with reducing chemicals, and they thereupon break up into smaller particles and lose their fibrous structures.

Of considerable importance is the observation that the changes in the yolk and white are both brought about by the same agent. This probably means that the deteriorations of the yolk and white on storage have the same cause and mechanism. The probability that

effect of reducing chemicals on the proteins is a splitting of the disulfide bonds points to the possibility that storage deteriorations may be caused by splitting of these bonds by other means as well as chemical reduction. It is known that disulfide bonds are also slowly attacked by alkali. Finally, chemical reduction itself may be involved in the deteriorations occurring on storage. Reducing groups actually exist in comparatively large amounts in egg white, but they are "masked" or very poorly reactive.

These results suggest that absorption of reducing chemicals through the shell causes eggs to deteriorate. Deteriorations were found to occur when eggs were stored in atmospheres containing very low concentrations of hydrogen sulfide and sulfur dioxide. Serious deteriorations occurred at concentrations of hydrogen sulfide below the lowest level at which many people are able to detect the sulfide by odor. Oiling the egg effectively retarded this absorption. Thus, the practice of oiling, long employed to reduce deterioration by preventing materials from leaving the egg (i.e., the loss of water and carbon dioxide), may be equally useful by preventing adsorption of reducing gases by the egg. However, the economic importance of deteriorations caused by exposure to reducing gases remains to be investigated. A critical survey is needed of the concentrations and amounts of reducing chemicals to which eggs are exposed during handling and storage.

Antioxidant Improves Frozen Creamed Turkey

The development of rancidity in frozen creamed turkey is known to occur in varying degrees. Ordinary methods of preparation are not adequate for complete prevention of this change in quality. Antioxidants suitable for use in foods have become available in recent years and their wider use may become legally permissible soon. Since little information is available on the use of antioxidant during cooking of foods such as turkey, experiments have been conducted at the Western Regional Laboratory to supply such information.

The use of a small amount of a food-grade antioxidant in the cooking water has produced cooked turkey that has no detectable rancidity immediately after cooking and that has little tendency to develop rancidity during frozen storage, as in creamed turkey or turkey a la king. Turkey fat rendered during the cooking process was used in the creamed turkey and, unlike fat recovered in the absence of antioxidant, appears stable enough to be used in other food products also.

The turkeys used in the experiments were cooked by simmering in water in a covered container; antioxidant was added to the water at the beginning of the cooking process. Half of each turkey was cooked with antioxidant and the other half without it. The cooked turkey meat was then removed from the bone, diced, and added to sauces consisting of turkey fat, broth, flour, and milk. Comparisons were also made with samples of creamed turkey to which the antioxidant was added after the birds were cooked.

Rancidity development during cooking of turkey and during storage of frozen creamed turkey was measured by peroxide determinations and by taste tests. In one series of experiments, peroxide values for turkey cooked without antioxidant ranged from 8 to 43, whereas samples cooked with antioxidant had values from 1 to 4. The variation in turkey cooked without antioxidant appears to be due to inherent differences in the birds. Rancid flavor was not detected in creamed turkey prepared from halves cooked with antioxidant, but with some lots of birds creamed turkey prepared from the halves cooked without antioxidant tasted rancid even before it was subjected to frozen storage.

The development of rancidity is also retarded when antioxidant is added during preparation of sauce for creamed turkey, but it appears more advantageous to add antioxidant during cooking of the bird in order to prevent the initiation of rancidity. An antioxidant mixture composed of 20 percent butylated hydroxyanisole, 6 percent propylgallate, 4 percent citric acid, and 70 percent propylene glycol was used in most of this work. It was ordinarily added to the cooking water at a level of 0.005 percent based on weight of the bird. Storage of creamed turkey in ordinary packaging material for 12 months at 0°F. revealed no significant increases in rancidity as determined by either flavor score or peroxide value when antioxidant was present, but significant increases were observed by both tests when no antioxidant was present.

Salmonella Microorganisms as Factors in Food Poisoning

Recent investigations have made significant new knowledge available on the part that Salmonella species play in food poisoning. The complex nature of the problem is evident from the fact that there are over 150 recognized species or types of Salmonella and that over 50 of these have been implicated specifically in food poisoning outbreaks. Actually, all species of Salmonella are by definition pathogenic for either animals or man or, of course, for both. It is presumptive that the remaining 100 or so species are pathogenic for man, since no species has been exonerated as a human pathogen.

Studies of the pathogenicity of Salmonella and further characterization of the clinical aspects of Salmonellosis resulting from oral feeding of known numbers of Salmonella have been conducted at the University of Chicago under a research contract. Twelve strains representing 6 species were used. All but one of these strains had been isolated from dried whole egg and were subsequently preserved in the dry state to avoid any possibility that their virulence might change as a result of repeated subculturing on artificial media. They were S. pullorum, S. meleagridis, S. anatum, S. newport, S. derby, and S. bareilly. Since all of these species (as isolated from various sources) had been implicated previously in food-poisoning outbreaks, it was anticipated that they would be found to be pathogenic at some dosage level. The study has provided information on

the dosage levels for oral feeding that cause overt illness and has provided valuable information on the clinical manifestation of Salmonellosis arising from oral feeding.

The study was conducted with human volunteers of a penal institution. Various numbers of viable Salmonella were placed in eggnog and fed to the volunteers, who were subsequently observed for manifestation of illness. Suitable control feedings were, of course, a part of the experimental design. Generally 6 men were fed at any selected dosage. The lowest dosage (number of microorganisms) causing overt illness of at least one man in 6 was 125,000. The numbers required to cause illness varied with the species and the strain. In one case as many as 1.3 billion microorganisms per dose were required to cause one man in 6 to become ill. Since the number causing illness varies with the strain as well as the species and undoubtedly with the age and state of health of the subject, the number limits must be considered illustrative rather than definitive. It was shown that in a number of cases the volunteers became carriers even though no illness was apparent. All cases of illness recovered and all carriers became negative after several months.

In spite of the rather widespread occurrence of Salmonella, citation of Salmonella as a cause of illness is fairly limited. This perhaps reflects the general rule that many cases of food poisoning go unreported if the illness is mild, as it may be in many cases of Salmonellosis.

Nevertheless, this study of the detailed pathogenic characteristics of 12 strains of Salmonella, when considered along with information available from other sources, emphasizes the view that more attention should be given to the control or elimination of Salmonella during the processing and preparation of food, particularly animal products. This Bureau, other research institutions, and industry are now intensifying research activities on methods of minimizing the occurrence of Salmonella in foods and producing in this respect better food.

Improved Sauces and Gravies for Frozen Precooked Foods

The curdling of sauces and gravies that occurs when frozen precooked foods are thawed affects the appearance of the product. To make the sauce smooth and attractive when frozen, chicken a la king, for example, is served, the housewife usually stirs it vigorously and although she improves the smoothness of the sauce, she breaks up some of the solid pieces of chicken and vegetable. A sauce requiring no stirring would be a decided advantage in the commercial development of precooked frozen foods.

Although experiments have shown some improvement in the frozen storage stability of sauces made with several of the 60 different starches, flours, and stabilizers tested, waxy (glutinous) rice flour has been outstanding in all respects. In flavor and appearance, unfrozen sauces prepared with waxy rice flour are difficult to distinguish from those prepared with wheat flour. When sauces thickened with the waxy rice flour were stored for more than 10 months at 0°F., they could not be distinguished from the freshly prepared unfrozen product. With wheat flour, the separation was observed even when the product was thawed immediately after freezing, and it became progressively worse with prolonged storage.

The investigation has revealed that the type of thickening agent is the principal factor in development of curdled appearance and liquid separation in sauces and gravies subjected to freezing and frozen storage. Thus amylopectin starches and flours minimize these defects but since amylopectin starches, in contrast with waxy cereal flours generally, have a "long paste" character, the starches themselves do not appear suitable for use in sauces and gravies. Flours from waxy cereals such as corn and sorghum yield sauces with improved stability, although the improvement is not quite as great in some respects as is obtained with waxy rice flour.

The temperature to which sauce is heated following freezing influences the appearance and liquid separation. Ordinary sauces thawed at room temperature shortly after they were frozen show marked curling and liquid separation, and these defects are accentuated during frozen storage. Heating of thawed sauces made with ordinary flour does not completely eliminate the defect in appearance and liquid separation, although both of these factors are decreased. Even sauces made with the waxy cereal flours thawed at room temperature may show considerable liquid separation when subjected to extended frozen storage (particularly if the temperatures are as high as 10 to 20°F.). However, when these sauces are heated, the liquid is completely taken up by the opaque phase even without stirring, and a perfectly smooth sauce results. These defects in appearance of sauces and gravies can be considerably reduced by using mixtures of wheat and waxy rice flour, particularly when a sauce is homogenized before freezing.

Toxic Action of Phosphate Insecticides

Recent studies in the Western Regional Research Laboratory have extended previously established knowledge of the inactivating effect of diisopropyl fluorophosphate on the digestive enzyme chymotrypsin. Studies with certain analogous esters of phosphoric acid (also with potential uses as war gases or insecticides) have shown that they also react with chymotrypsin. The substances selected for testing represented a variety of types of these phosphate esters. In every case the enzyme took up only one phosphate group per molecule of enzyme (which is the reaction previously described) yet that very small change was sufficient to inactivate the enzyme completely.

While the reaction was shown to be the same in each case, the speed with which it took place varied very much from one inhibitor to another. The most active inhibitor tested (diisopropyl fluorophosphate) reacted about 1000 times faster than the slowest one tested. Since the reaction is the same in each case, it would appear that the most poisonous must be so because they react the most rapidly. Measurements were made on the following compounds, which are listed here in order of decreasing activity: diisopropyl fluorophosphate (a potential war "gas"); tetraethyl pyrophosphate (an insecticide); diphenyl chlorophosphate (a phosphorylating reagent); diethyl thionofluorophosphate; tetraisopropyl pyrophosphate; diethyl p-nitrophenyl phosphate; diethyl p-nitrophenyl thionophosphate (the insecticide "parathion"); tetrapropyl dithionophosphate.

Thus a wide variety of insecticidal phosphates share the property of reacting with chymotrypsin. There is also good reason to believe that the same reaction occurs with other vital enzymes, and underlies the toxicity of the so-called "nerve gases". Investigations have shown that trypsin undergoes exactly the same reaction, while from other laboratories (notably in England) evidence is forthcoming that the important brain enzyme, cholinesterase, whose inactivation is so promptly fatal, behaves in all probability as do trypsin and chymotrypsin.

Now that the chemical mechanism of the poisoning is known, its reversal, or detoxification, becomes an important objective for those who are searching for antidotes and protective drugs. Such reversals have not yet been satisfactorily accomplished in the laboratory, although we have observed that some plants, notably citrus, seem to possess a mechanism which reverses the inactivation in some cases, though not in all.

SUGAR AND SPECIAL PLANTS UTILIZAYION INVESTIGATIONS

The Organic Acids of Sugar Beets

More adequate knowledge of the composition of sugar beets may make possible removal of materials that interfere with the crystallization of sugar. Some non-nitrogenous organic acids account for a large portion of the non-sugar soluble solids (as revealed by earlier studies). The Western Regional Research Laboratory has studied the separation and analysis of these acids by two new chromatographic methods.

In one method (paper chromatography), solutions containing an amount of acidic material too small to be weighable on the ordinary analytical balance are placed near one edge of a sheet of filter paper. An organic solvent capable of dissolving the acid is allowed to diffuse from that edge to the other end of the paper. The acids move a distance which depends upon their solubility in the applied solvent. After diffusion, the paper is dried

and the organic acids located with acid-base indicators. The distances moved by unknown acids are compared to those for knowns. When they are the same for a given pair of acids with 2 or more organic solvents, it may be assumed that they are the same.

The next step, which is accomplished on ion exchange resins, is to isolate larger amounts of the tentatively identified acid. A portion of strong anion exchanger is adjusted to an acid concentration which is satisfactory for separation of weak acids, and another portion adjusted for strong acids. The second portion is placed in the lower part of a column, the first in the upper part of the same column. Sugar beet juice pretreated to remove nitrogenous compounds is adsorbed on the column. It is then slowly eluted with a dilute solution of strong acid. As liquid flows from the column it is collected in a series of small samples. Each sample is tested by paper chromatography. All these samples containing the tentatively identified acid are combined. Isolation of pure acid from these fractions is readily accomplished. The combination of these two techniques has been so successful that nearly all the organic acids in sugar beet juice have been separated in pure form and only trace amounts of some acidic impurities remain to be identified.

The major organic acids identified and analyzed in sugar beet juice are lactic, citric, malic, and oxalic acids, with pyrrolidone carboxylic acid appearing in processing liquors. Glycolic, glyceric, and succinic acids are present in small quantities. Through the cooperation of the beet sugar industry processing liquors were obtained from 9 sugar beet factories located in the principal sugar beet growing areas. Examination of these materials shows that only citric and oxalic acids are removed by currently used processing procedures. Consideration is now being given to ways of removing more acids so that yield of sugar can be increased.

The chromatographic procedures developed for the organic acids of sugar beets should be applicable in studies of composition of other agricultural commodities, fermentation liquors, and biological fluids.

STRATEGIC AND CRITICAL AGRICULTURAL MATERIALS

Continuous Milling of Guayule and Deresination of Guayule Rubber

At the U. S. Natural Rubber Research Station, Salinas, Calif., investigations on extraction of rubber from guayule have continued, as part of a broad program of preparedness against loss of Far Eastern supplies of natural rubber. This work is authorized under the Critical Materials Stock Piling Act of July 23, 1946.

Continuous Milling of Guayule Shrub: An improvement in the pebble-milling process for recovering rubber from guayule has been described previously. Briefly, this consists of processing freshly harvested shrub in contrast to shrub that is "conditioned" by field exposure or prolonged storage. Development of a means of processing shrub immediately after harvest has important advantages in that it minimizes or prevents degradation of rubber due to exposure to sun to prolonged aging. The new process also simplifies and lowers cost of shrub handling, results in higher over-all yields, and yields a crude rubber of greater uniformity as well as superior quality.

Further improvement in pebble milling has subsequently been demonstrated by the installation and operation of a continuous tube pebble-milling circuit in the pilot plant. This installation embodies a number of advancements over the process used commercially and by the Emergency Rubber Project during World War II.

The new circuit includes a controlled feed mechanism for introducing crushed guayule at a predetermined rate into the tube mills, improved rectangular flotation tanks with provision for continuous removal of bagasse, and an automatic high-pressure decorking unit called a "baica". Two tube mills, 27 inches in diameter by 12 feet long, are used in series for the milling proper. Two jordans (machines used in the paper industry) are included in the experimental plans (though not yet in operation) to investigate the replacement of one or both tube mills with a more efficient device.

Guayule factories have had high labor requirements. The present pilot plant represents not only mechanical improvement but also significant saving of labor at several points. A notable example is the automatic baica, which eliminates the need for two men formerly required to charge and discharge batch equipment. The baica effects an almost instantaneous waterlogging of cork fragments which accompany the rubber worms from the primary flotation, through hydraulic high-pressure treatment. Previously this was accomplished by means of the low pressure "paila" that required 40 to 60 minutes' treatment time as well as additional labor. Other savings in labor have been effected in shrub treatment before milling and milling operations in general.

Freshly harvested shrub is being processed. Throughput is approximately 150 pounds per hour (defoliated dry-weight basis). Extensive millings have been conducted in conjunction with a deresination operation, primarily to produce sufficient rubber for industrial fabrication of experimental truck and passenger-car tires. The purpose is to determine whether deresinated guayule rubber is good enough to serve as a supplement to, or replacement for, Hevea rubber in critical uses. Approximately 3,000 pounds of deresinated rubber have already been distributed to rubber companies. Tires from this rubber will be evaluated by the Office of Rubber Reserve test fleet.

Improvement of Rubber Quality Through Deresination: Processing improvements, including milling of freshly harvested shrub and deresination, have yielded rubber believed to be superior to any produced by previous methods. Crude rubber as ordinarily recovered from pebble milling contains around 20 percent or more of resins. This gross impurity limits usefulness of the rubber and contributes to lack of uniformity and to poor aging qualities on storage. Therefore, intensive efforts are being made to develop a practicable process for removal of the resins. One experimental method consists of extracting the comminuted shrub with acetone before milling. This yields a crude rubber of very low resin content, provided about 65 percent of the total shrub resin has been extracted. The other method is an extraction of resinous rubber agglomerates as normally milled. Tests indicate that the two procedures can be made to yield deresinated rubbers that are essentially equivalent in physical properties.

During the past year a batch method was developed and applied on a semi-pilot-plant scale for deresinating crude rubber in the form of small agglomerates (commonly called "worms"). This procedure offers a processing advantage over deresination of shrub because a much smaller weight of material is processed. Another advantage is that worm-deresinated rubber can be dried more readily and at low temperature, because after deresination the rubber is wet only with acetone, a readily volatile solvent. With shrub deresination the final product is normally wet with water, since acetone extraction takes place before pebble milling. High drying temperatures, as required for evaporating water, have been found to be deleterious to rubber quality. The rubber worms present more difficult problems of handling in the solvent extraction system, however, and these must be solved before a satisfactory process can be developed for factory operation.

The presently developed method of worm deresination consists essentially of placing the wet mass of rubber crumbs in a screened basket supported in a vessel to which 3 pounds of acetone are added for each dry-weight pound of rubber. The worms are dispersed into a slurry within the basket and extraction is facilitated by constant stirring. After about 3 to 5 minutes the miscella is withdrawn and extraction is continued by adding successive portions of fresh acetone or of partially saturated miscella from a previous run until the residual resin content is reduced to a minimum, usually within the range of 1.25 to 2.0 percent. The early part of the extraction removes water primarily; later the resins are progressively solubilized. A hot extraction was found slightly more rapid than a cold, but a comparison of rates and completeness of extractions between runs made at 30°C. and 50°C. showed no practical advantage for the higher temperature. Batches of about 35 pounds of rubber worms are deresinated in the semi-pilot-plant equipment. The extraction system comprises a total of 16 separate leaches with acetone, which are conducted in countercurrent fashion. Antioxidant is applied in an acetone solution after deresination; then the rubber is spread out on trays for drying. By this method large experimental lots of deresinated rubber have been produced for evaluation in truck and passenger car tire manufacture.

EASTERN REGION
P. A. Wells, Director

FRUIT AND VEGETABLE UTILIZATION INVESTIGATIONS

Apples and Other Fruit Utilization Investigations

Juice Products

Apple Concentrate

One of the problems involved in preparing a high quality apple concentrate is to develop a practicable method of preparing a light colored product. Decolorization of apple concentrates with activated carbon did not prove to be efficient. Experiments have been carried out therefore on decolorizing the apple juice before concentration. This proved to be much more effective. Dosages of from 0.1% to 0.4% activated carbon on the basis of the juice gave effective decoloration. A temperature of 70°C. and a time of 20 minutes gave optimum results. The carbon treatment could be carried out on a freshly-depectinized juice before filtration with little loss in efficiency. At the completion of the Pectinol treatment period, the juice is heated and the carbon added. The heating thus serves the double purpose of inactivating the Pectinol and increasing the efficiency of the carbon treatment. A single filtration removes both the carbon and the precipitate from the Pectinol treatment, yielding a sparkling clear, almost water-white filtrate. This filtrate is evaporated under vacuum, yielding a very light-colored concentrate.

Bland Apple Sirup

A commercial demand has developed for bland apple sirup as a humectant in the cigarette industry due to the rapid increase in glycerine price and a possibility of serious curtailment for this use. Various apple sirup samples were made to determine whether the levulose content could be improved and whether sirup could be made at a smaller cost from apple concentrate and in equipment normally available to concentrate manufacturers. All of these goals were achieved. Samples and analyses were distributed to cigarette manufacturers for evaluation. The method for preparing it from the concentrate is important, as it would permit its preparation in case a demand for it arose in seasons when apples were not available.

Full-Flavored Fruit Concentrates

Studies on the use of essence in two types of Concord grape juice concentrates have been continued. The first is the full-flavored, or self-preserving type, of 70° Brix. The storage test has been continued to determine the keeping properties at 70°F., 35°F., and 0°F., of the regular concentrate; of the concentrate without essence; and of the combined "first" and "second" essences stored separately. No significant deteriorations in flavor have been observed except in those samples stored at 70°F.

The other type is the frozen concentrate of about 42° Brix. A new process, completing the concentration of the juice during the ultra-rapid atmospheric vaporization used to strip off the essence, was developed. This was facilitated by the short retention time characteristic of our improved essence recovery unit which reduces heat damage to the juice. This process is of special interest in the case of frozen, sweetened fruit juice concentrates, in which a high degree of concentration of the juice is not required. Concentrate of 47° Brix was prepared by evaporating the juice to 35° Brix and then adding sugar in an amount almost equal to the grape solids in the juice. Some commercial grape concentrates are understood to contain approximately this amount of added sugar. This degree of concentration was conveniently accomplished in the essence recovery equipment simultaneously with the removal of the volatiles for recovery as essence. In a similar manner there was also prepared an unsweetened concentrate of about 47° Brix without damaged flavor.

Samples of sweetened and unsweetened concentrate made by the above process and also by the double-essence process recently published were of excellent quality after two months' storage at 0°F. These tests will be continued up to a year's storage.

Studies are being made on frozen apple juice concentrates prepared by various processes. The apples used in preparing the juice had been in cold storage for at least six months. They were sound, but on account of the long storage period they were not necessarily typical of the apples customarily used for juice production. The processes employed were the return of essence to a concentrate as usual, and the addition of fresh juice instead of essence. The effects of heat treatment, depectinization, filtration, degree of vacuum used in concentration of the stripped juice, as well as various storage temperatures for the frozen and unfrozen products are being studied to determine the initial and keeping qualities of the products. Results to date indicate that pasteurization of the juice is achieved during the essence removal, and that for the apples used further heat treatment as used in conventional 180° pasteurization has a deleterious effect. With these apples, concentrates made at 27" vacuum (115°-117°F.) were inferior to those made at 28.6" (88°-90°F.).

Volatile Flavor Recovery and Applications

Production and Outlets

While the effect of the Internal Revenue tax situation is still felt by the essence industry, the commercialization of this development is progressing at a rapid rate. With the industrial expansion of the frozen fruit juice concentrates to other than citrus fruits, there has been an active interest in this application of the essence development, especially as the quality of most of the present frozen apple concentrates has been reported to be unsatisfactory. A fruit products broker states there is a great opportunity for the production of frozen full flavor concentrates (using the essence feature) from a wide variety of fruits for use in jellies, jams, etc.

An Ohio fruit processor who had installed an apple essence recovery unit several years ago is making a wide distribution of full-flavored apple and grape juice concentrates. Both are reported to be of excellent quality. Recently they are understood to have extended their operations to include strawberry and cherry full-flavored concentrates.

The Quartermaster Food and Container Institute reported successful results with 350-fold apple essence, obtained from a commercial source, as a flavor in a powdered food product. This essence had been prepared from commercial 100-fold essence.

Processing Cherries

Work to improve the quality and increase the yield of processed cherries was continued. One phase of this work was an intensive study of the processing of red cherries under rigidly controlled laboratory conditions. The outstanding features of the results were: (1) no loss in quality of the cherries occurred during storage for 3 to 4 weeks at 35°F., (2) both the overall yield and drained weight increased significantly during storage, (3) yield and drained weight were increased greatly by storage of the cherries for a short time at 75°F., and (4) bruising and brief storage of the cherries at 75°F. gave the greatest increase in yield and drained weight.

Since it is thus clear that red cherries may be stored under refrigeration for 3 or 4 weeks without suffering loss in quality, it may be possible to lengthen the normally short processing season. Also, proper treatment of cherries may increase the yield of processed product by 5 to 10 percent.

A study was also made to determine where bruising occurs during the commercial picking and handling of cherries. Cherries are bruised at each stage of handling: during picking, during hauling, and after soaking. A survey made in one large processing plant showed that the cherries were dropped from flumes to tanks, from conveyors to belts, and from belts to belts at least 17 times, the height of each drop varying from 6 inches to 3 feet, with a total cumulative distance of over 21 feet. The excessive bruising which results defeats the principal purpose of soaking, which is to facilitate pitting and reduce pitting losses.

Potato and Other Vegetable Utilization Investigations

Processing of Vegetable Wastes

Further Feeding Trials

Approximately 400 pounds of broccoli leaf meal prepared in the commercial vegetable waste dehydration plant at Seabrook Farms in New Jersey were turned over to the Delaware State Experiment Station for testing as a source of pro-vitamin A and xanthophyll in large scale energy broiler feeding trials. Soybean meal and corn were the major ingredients of the diet with suitable amounts of vitamin and mineral

concentrates to give a complete diet when either broccoli leaf meal at 1.5, 3 or 5 percent levels or alfalfa at a 5 percent level were added as sources of carotene and xanthophyll.

As in previous feed trials 1.5 percent level of broccoli meal was equal to 5 percent alfalfa meal in all respects. Three and 5 percent levels were only slightly better in weight production, but color and general appearance of the birds were better. The average weight of the birds on these high energy rations was 2.97 pounds at 10 weeks.

It is the opinion of the Delaware group that 1-1/2 or 3 percent broccoli meal could be used to great advantage in these diets and that a favorable price ratio could be established for the higher value broccoli meal.

A second feeding trial on the comparison of extracted broccoli meal and soybean oil meal as protein supplements in broiler rations has been completed. As in the previous trial the broccoli fed birds grew in a perfectly normal manner but were lighter in weight than the soybean fed group. Professor Runnels of the Delaware Agricultural Experiment Station believes that this may well be due to the bulkier nature of the extracted broccoli leaf meal when used in the high concentration of approximately 50 percent of the diet. He has suggested pelleting of the material as a possible solution, and this will be tried in new feed trials this fall as soon as new broccoli leaf meal is available.

Rutin

Preparation of Soluble Rutin Compounds

Numerous requests have been received from physicians and pharmacologists for a water-soluble form of rutin which might be used therapeutically for intravenous injection. However, no satisfactory soluble form of rutin exists to make the drug quickly available to the capillaries. In the usual oral administration it must be solubilized and absorbed through the digestive tract. This is a comparatively slow process.

A number of flavonoid water-soluble compounds have been prepared. Solutions of rutin with ferrous gluconate and of rutin with saccharated ferric oxide now appear to be the most promising. These compounds solubilize rutin to the extent of about 200 times its solubility in water. Preliminary tests indicate that both of these preparations can be administered either orally or intravenously. Very recent literature presents accounts of the successful intravenous injection of solutions containing either ferrous gluconate or saccharated ferric oxide for treatment of lypochromic anemia. These metallic complexes of rutin show promise of important medical applications.

Production of Rutin

The use of rutin in clinical medicine has been encouraged as opportunity arose. The general availability of rutin in drug stores

has led to its widespread use, but has made it difficult to estimate the volume of the drug passing through commercial channels. Interest in the use of rutin has been increasing among European physicians judged by the growing number of articles on the drug that appear in European medical journals. During the past year there have appeared 5 such articles in German medical journals, 2 in French, 2 in Czechoslovakian, and one each in Italian, Norwegian, Brazilian and Egyptian. Since the last report 8 papers on the clinical use of rutin or vitamin P have been noted in American medical journals.

Manufacturers report that sales of rutin show a constant increase.

Feeds from Vegetable Wastes

Seabrook Farming Corporation Contract

Experimental production of several dried leaf wastes was undertaken during the 1950 growing season by the Seabrook Corporation. Trouble with the commercial drier slowed down operations at first, but when the drier was revamped according to designs supplied by the Laboratory, the trouble was overcome. During the season, 6 tons of pea vine meal and 30 tons of broccoli leaf meal were produced. The broccoli meal is high in protein (36-41 percent) and vitamin A (300,000 to 400,000 I.U. per pound). As such it is a good broiler ration. The high chlorophyll content of the broccoli meal, however, makes it more valuable as a source of chlorophyll, and a chlorophyll producer has purchased broccoli leaf meal from Seabrook and is now using it for chlorophyll production.

Tomato Cannery Waste

A method was developed for drying tomato cannery waste press cake in a direct-fired rotary drier of the type commonly used for alfalfa. The waste from tomato juicing operations was first pressed to a moisture content of about 68 percent. At this moisture content the cake could be fed directly to the drier. Drying was effected with inlet gas temperatures of 870°, 1055°, and 1200°F. The 870°F. product, reported one feedstuff manufacturer, is superior to any similar product on the market, the 1055° product was equal or better than any now manufactured, and the 1200° F. product was slightly scorched. Feeds made from tomato waste could be used to augment the supply of foods that might be critically short in times of national emergency. It is believed that they could be largely used in the areas where they are produced and thus alleviate the problem of transporting feedstuffs into these areas.

Potato Utilization Investigations

Industrialization of Allyl Starch

Previous reports have described progress made in the development and commercialization of allyl starch containing about 1.8 allyl groups per glucose unit. Although starch itself is insoluble in almost every solvent, freshly made allyl starch is readily soluble in a number of organic solvents. When dissolved, it can be brushed or sprayed on wood, glass or metal just like any other lacquer. After

the solvent evaporates, a slow chemical reaction involving the oxygen in the air takes place in the film which makes it insoluble in organic solvents and impervious to oils. The surface retains its high gloss and becomes hard and resistant to temperatures up to 400°F. Allyl starch has been used for furniture finishes, interior wood coatings, metal finishes, heat-setting adhesives, and as a printing ink vehicle. The printing ink industry uses the major portion of the present allyl starch production.

Current laboratory work is concerned with the development of aqueous emulsions of allyl starch in order to avoid the cost and fire hazard involved in the use of organic solvents. Attempts are also being made to formulate allyl starch pastes which can be diluted with water for emulsion applications. Several emulsion recipes have already found commercial application. Some progress has also been made in reducing the drying time of allyl starch films. For example, ultraviolet irradiation produces the same degree of hardness in 4 to 6 hours that is normally obtained in 24 hours.

Pilot plant production of allyl starch was announced by General Mills, Inc., in 1947. Since that time there has been a steadily increasing demand for allyl starch which has required periodic increases in production facilities of the producer during the past 4 years. At present they are installing new production facilities to meet increased demands for allyl starch. Perhaps the greatest potential value of allyl starch is as an extender or replacement for shellac in the event that imports of the latter are interrupted.

Fermentation of Potato Starch Factory Wastes

The extracted pulp and processing water of potato starch factories are discharged into streams because no recovery methods have yet been accepted by industry as being economical. Health authorities have become critical in recent years of industries that dump their waste products into streams. Such wastes require oxygen for the conversion to stable forms, the oxygen being removed from the water at the expense of aquatic life and at the same time lowering the potable value of the water. In Maine the health authorities have banned the establishment of new potato starch factories on sites not previously occupied by starch plants.

The Eastern Regional Research Laboratory has conducted studies on the composition and properties of these wastes in the past and has made a cost analysis on pulp recovery. It was recommended that the pulp be recovered for livestock feed, and a pilot plant has been operated during the past two years by the Maine Institute of Potato Starch Manufacturers at Mapleton, Maine, for this purpose. Even if the pulp recovery process proves successful, there is still no economically satisfactory method for recovery of the solubles from the dilute processing water by mechanical or chemical means. Studies at the Eastern Regional Research Laboratory have shown that removal of protein by precipitation methods leaves more solubles than can be neglected. It was therefore decided to carry out experiments in microbiological treatment of the wastes.

The object in this study was to develop a process in which the mixed potato starch factory wastes could be fermented so as to convert the soluble organic material into an insoluble form. The insoluble substances could then be separated by filtration or centrifugation, leaving an effluent low in Biochemical Oxygen Demand (B.O.D.). The solids might be used for feed or possibly fertilizer.

Several types of fermentation were tried on the total starch factory wastes. A combination of microbiological treatment and acid precipitation of protein gave the lowest C.O.D. values of all. Two samples of waste protein water (original C.O.D. 4300 units) were used that had been fermented using "back set"inocula from a continuous fermentation initially inoculated with the soil culture. Acidification of one fermented sample reduced the C.O.D. from 1100 to 340 and the other from 860 to 190 units.

If 100,000 gallons of daily waste from a starch factory had to be heated from 5° to 30°C. and maintained at that temperature during treatment, heating costs alone would be a major item.

The above fermentations fortunately, however, evolved such large quantities of heat that it was necessary to cool the fermentor. The availability of cold surface and well water in Maine and Idaho during the operating season should make cooling an economical step.

Conversion of Surplus Potatoes to Stable Form

A new method of drying potatoes was developed which employs a direct-fired rotary drier commonly used for drying alfalfa. The process, briefly described, consists of the following steps. The potatoes are washed and then ground in a hammer mill. Some coarse dried potatoes are mixed with the freshly ground material to avoid sticking in the drier, and the mixture is passed through the drier. Before any of the dried material is recycled for mixing with the freshly ground product, it is passed over a 100-mesh screen to remove the fines and reduce the hazard of dust explosion. The product includes the material which passes the screen and that portion of the coarse material not required for recycling.

Cost estimates show that a plant processing 62 tons of potatoes to make 13.8 tons of product in 24 hours would require a capital investment of about $110,000. The cost per ton of product, including everything except the cost of the potatoes and the selling costs of the product, would be $34.00 a ton.

Natural Freeze Drying of Potatoes

The experiments on Maine potatoes, started as described in last year's report, were encouraging. Potatoes that had been spread on the open ground at Presque Isle in February were examined in mid-June, 1950. They were partly dehydrated (45 to 55% moisture, as against an initial 80%) as the result of alternate freezing and thawing. They were virtually free of decay. Potatoes spread in mid-April were not exposed to sufficiently severe freezes to bring about dehydration.

The field-dried potato product is under study as livestock feed and as a source of alcohol.

Nutrient losses in natural freeze dehydration are evidently large, but nitrogenous and mineral substances passing into the soil stimulate pasture growth tremendously. A heavy stand of grass grew through 4-inch and 8-inch layers of potatoes in the spring of 1950.

Potatoes were also freeze-dried under controlled laboratory conditions. The potatoes were of uniform size (medium) and density (1.045). Over 3 weeks they were alternately frozen and thawed for 9 cycles; the weight decreased 45 percent. After this three-week period, they were left at room temperature for 35 days; the total decrease in weight was 83 percent. The potatoes contained originally 84.4 percent moisture and at the conclusion, 18.4 percent. The 15.6 pounds of solids per 100 pounds of potatoes was reduced to 13.9 pounds, a loss of 11 percent.

Samples taken at intervals during the drying period were studied under a dissecting microscope. The following tentative conclusion was made: When a potato is frozen its tissues expand; this expansion is greater or faster in some regions than in others. As a result, in many regions the intercellular cement gives way, and groups of cells are sheared from other groups. For the most part no cells or cell walls are broken, and the starch remains within the cells.

When thawing occurs, sap diffuses from the cells, for the differential permeability of the cell protoplasmic membrane is destroyed by freezing. The sap moves into the empty space formed by the shearing apart of the cell clusters. This results in a continuous network of channels filled with cell sap. When the network comes in contact with permeable parts of the potato skin, such as occur near the eyes, lenticels and bruised areas, sap is lost and the tissues contract.

Most of the change in tissue structure occurs during the first freeze.

SUGAR AND SPECIAL PLANTS UTILIZATION INVESTIGATIONS

Tobacco Investigations

Nicotine Synergists

The use of nicotine as an insecticide is declining rapidly due to competition from organic insecticides. Since synergists offer the greatest promise both for reducing the cost of using nicotine and increasing its general effectiveness, this field has been actively investigated in cooperation with the BEPQ and the Connecticut Agricultural Experiment Station; 600 compounds have been screened. Mr. Neely Turner, of the Station, has found that 6 of the 34 polyethylene glycol esters and ethers supplied by this Laboratory increase the toxicity of nicotine to aphids by 5-7 times, 2 increase the toxicity 10 times and 3 increase it more than 10 times. The latter group comprise the monolaurates of polyethylene glycols 400 and 600 and the monooleate of polyethylene glycol 1000. These compounds are essentially non-toxic to aphids by themselves.

As a result of these preliminary experiments on aphids, about 15 commercially available polyethylene glycol esters were obtained and forwarded to the BEPQ station at Anaheim, California, for testing.

In addition, samples of isophthalonitrile, two additional commercial nitriles, about 20 anthraquinone derivatives, bis-(2-aminophenyl) disulfide, p-toluyl-o-benzoic acid, and 2-methylanthraquinone were sent to Anaheim for testing.

Since some 12 compounds have now been found which are unquestionably synergistic with nicotine as insecticides, there seems to be a good chance that some of these will survive field tests and will be used with commercial nicotine preparations.

To promote this development further, a compilation of the more effective nicotine synergists has been prepared, listing the physical and chemical properties of the compound together with the known toxicological data. This information has been made available to several division heads of BEPQ. It was decided that this report would be distributed throughout the field and that the program for the coming year would include the possibility of nicotine synergist testing.

Tobacco Essence

Last year's report described a method for recovery of tobacco essence from low grade tobacco and tobacco stems, and the interest of the trade in utilizing the essence for enhancing the aroma of some of the lower grade tobaccos and for imparting a tobacco aroma to packages for tobacco products. A relatively large amount of tobacco essence was prepared and distributed to the trade for evaluation. Results of these tests have developed an interesting lead in an unexpected direction. When cigar leaf tobacco is stored it sometimes gets infected with a mold. The mold imparts a grey color and musty odor to the tobacco, which is usually destroyed. Spraying with tobacco essence, however, restores the normal brown color and masks the odor, so the tobacco can be used.

Tobacco Stem Technology

Following requests from tobacco processors, a survey was made of the tobacco stemming industry in North Carolina and Virginia to determine how an increasing supply of stems might be utilized for agriculture. Within the past 2 years the tobacco industry has changed its methods of stemming tobacco. Formerly this operation was largely performed on old tobacco out of storage and just prior to its preparation for manufacturing. The stems were sent to the nicotine factories where they were processed and sterilized. This operation extended over the year.

Now the stems are removed from the tobacco as it is received from the farmers and before it is packed. It has crowded much of the stemming operation into 6 months, from August to January, and much of the work is now being done in the market towns in North Carolina and Virginia. The increased seasonal accumulation of stems in newly

established centers of production has suddenly developed into a serious agronomic problem. The stemmers are disposing of their green stems to the farmers and others in their vicinities, and thus are distributing virus and other diseases that such unsterilized material may carry. To solve the problem it will be necessary first to determine the conditions of heat, time, and moisture required for sterilization of the stems; and to determine whether this can be done economically in existing types of equipment.

Tanning Materials, Hides, Skins and Leather Utilization Investigations

Development of Domestic Tanning Materials

Investigations were continued at the Eastern Regional Research Laboratory on the development of additional sources of vegetable tannins. These included cooperative work with the Michigan College of Mining and Technology on the salvage and utilization of waste hemlock bark from lumbering and pulping operations; cooperative studies with the Tennessee Valley Authority on the salvage of oak bark from slabs; completion of the studies and publication of the report on the tannin content of two Florida scrub oak barks; and continued cooperation with the Bureau of Plant Industry, Soils and Agricultural Engineering on development of domestic sumacs, in which over 3000 pounds of sumac leaf produced in Beltsville, Maryland are being evaluated for tannin content and tanning properties, both as ground leaf and as tanning extract.

Samples of leaves, barks and wood from four species of trees--Conocarpus erectus, Rhizophora mangle, Avicennia officinalis, and Acacia macracantha--were received from the Minister of Agriculture in Caracas, Venezuela. Some part of each of these trees is now being used as a source of tannin and it was desired to know whether other portions could be mixed and used advantageously.

Of the Venezuelan materials studied, tanning extract might be made from a mixture of Conocarpus erectus wood and leaves but such extract might be subject to the difficulty of sediment formation on standing. Satisfactory tanning extracts may be prepared from the barks of Rhizophora mangle and Avicennia officinalis.

Improved Alum Retannage Procedures

In studies of alum retannage of insole leathers, it was shown at the Eastern Regional Research Laboratory that commercially available basic aluminum acetate could be substituted advantageously for the mixture of aluminum sulfate, sodium carbonate, and sodium acetate previously used. This substitution resulted in a reduction of the mineral salts in the leather and simplified the application of the retannage in the tannery.

A further improvement in the retannage method is the development of a dry dipping process. In this, dry, vegetable-tanned leather is dipped into a basic aluminum acetate solution of proper concentration (usually about 11 percent) for a period of about 30 minutes at

45°C. The leather absorbs the solution containing the aluminum acetate and retannage. Fixation of the aluminum oxide in the leather is accomplished while the leather is sammied (held in a moist condition) for 48 hours. After this treatment the leather is dried and finished.

In further studies of the application of the dry dipping method of retannage to cut insole leathers, 320 soles were cut from 40 commercially tanned bellies and retanned at the Eastern Regional Research Laboratory. Because these leathers differed in absorptive properties they took up different quantities of the tanning solution and were thus retanned to different degrees. The cut soles were divided into three groups according to their estimated aluminum oxide content: (1) up to 2.5%, (2) 2.5% to 3.0%, and (3) 3.0% and above. After wetting back, light reoiling and reworking to relieve excessive stiffness, 294 of the retanned cut soles were furnished to the Philadelphia Quartermaster's Laboratory for use in exposure tests under various atmospheric conditions.

The importance of the successful application of alum retannage to military insole leathers is evidenced by a statement of the Research Director of the Research and Development Branch, Textile, Clothing, and Footwear Section of the Office of the Quartermaster General (Leather and Shoes, October 28, 1950) - "Currently a specification is being prepared based upon a combination vegetable and metallic retannage. Some technical problems still remain to be worked out, but the prospect of our adoption of such a combination tanned insole, with particular emphasis upon alum as a retannage, should be faced by the industry, and further plant trials run voluntarily by individual tanners...."

Production of Canaigre

To complement the field work on the production of canaigre carried out by the Bureau of Plant Industry, Soils and Agricultural Engineering, wild canaigre roots from different regions were evaluated for tannin content, purity, and extractability. The tests showed that roots from southern California, southern Nevada, northern Arizona and southwestern Utah were highest in tannin content and purity; hence strains from these regions are most promising as planting stock.

BPISAE has plantings of canaigre at Plainview, Texas; Portales, New Mexico; and Elfrida, Chandler Heights, Higley and Yuma, Arizona, totalling about 19 acres which will be ready for harvest this year. These plantings should yield about 190 tons of roots, which are scheduled for processing into canaigre tanning extract.

Processing and Using Canaigre

Laboratory studies show that the tannin is more readily extracted by hot water when the starch in the roots is destroyed by fermenting the shredded roots with Aspergillus niger. This fermentation may be made continuous. The canaigre extract, which contains sugars, may in turn be continuously fermented with Aerobacter aerogenes and the sugars destroyed.

Canaigre tanning extract was used for laboratory tanning of heavy leathers. All the leathers prepared were well tanned, firm, suitably filled and of good appearance.

In a pilot plant study, 732 pounds of dried tannin extract were produced from canaigre roots.

The research and pilot plant results indicate that the manufacture of this domestic tanning material is commercially feasible. Normal production costs may be somewhat higher than competing imported materials, but would not be unreasonable if a national emergency cut off imports.

Sugars and Sirups Investigations

Honey Investigations

Continuation of the research at the Eastern Regional Research Laboratory on the utilization of honey is directed to both non-food and food uses. It is extremely important to provide as many market outlets for honey as possible so that the bee population will be maintained at a high level in order to insure adequate pollination of various crops.

In order that as much honey as possible may be withdrawn from hives, large quantities of sugar are fed to bee colonies at certain times during the season. This is true even though the darker, more strongly flavored, and less marketable types of honey, which can be used for this purpose, may be in surplus in some areas. A primary reason for this anomaly is that shipping this honey from one area to another may spread American foulbrood. In a study of methods of sterilizing this honey, attempts were made to combine the action of germicides and mild heat. Sterilization was achieved, but when the sterile honey was fed to bees a large number of them died. The attack on the problem was then shifted to the use of heat alone -- high temperatures for short periods of time. Fundamental data on the heat resistance of the spores of Bacillus larvae, the cause of the disease, have now been obtained and are being used to develop an inexpensive continuous process and to design equipment for this use.

An investigation of the potential place of honey in commercial baking is being undertaken by contract at the Kansas State College. A report covering the investigations on bread is in preparation. Other phases of the research include fruit cakes, yeast-raised sweet goods and several types of commercial cakes, where the valuable moisture-retaining properties and the desirable flavor possibilities should be of great value. It has definitely been confirmed that richer flavor, better palatability and superior moisture retention result when 40% of the sugar in a standard commercial yellow cake formula is replaced by honey. When honey replaced other sweeteners in standard commercial white bread it was detected by a majority of a taste panel even at the 3% level. Certain floral types of honey were shown to be more desirable than others for white bread manufacture.

Commercial fruit juice concentrates can be mixed with good-flavored honey and crystallized to give a fine-grained honey-fruit spread in which both fruit and honey flavors are present.

One of the difficulties in marketing liquid honey is its tendency to granulate to a semi-solid mass by the formation of crystals of dextrose, one of the honey sugars. Controlled heating to melt all possible "seed" dextrose crystals is the method used to check this tendency, but the absence of any practical method of controlling this heating has probably caused considerable overheating with consequent flavor damage to such honey. A device has been developed which is intended to facilitate routine examination of honey for the presence of these undesired crystals during processing and in storage. Based on the old principle of enhanced visibility of many crystalline materials by polarized light, the instrument permits ready detection of a single crystal in a jar of honey. Application of this method should result in better control of granulation in commercial packs.

Maple Products Investigations

In continuing work aimed at wider utilization of maple products, considerable attention was devoted to basic chemical studies of maple sap and maple sirup. Both sap and sirup have been separated into acidic, basic and neutral fractions. Fourteen organic acids have been found in both sap and in sirup, but only nine are common to both.

A preliminary study of the development of color in maple sirup indicates that the color of the finished sirup depends on the time of boiling the sap to sirup. The results show that production of light-colored, high-grade sirup will be favored by high evaporator efficiency (rapid evaporation), skillful operation and high sugar content of the starting sap. Evidence has accumulated that color and flavor are produced by reactions taking place during the heating of sap.

The new high-flavored sirup used in making blends, as described in last year's report, is now in commercial production by several processors.

A new maple sirup dial type thermometer has been developed with the cooperation of the Rochester Manufacturing Co. of Rochester, New York, and is now commercially available. This thermometer has an open legible scale much superior to those in thermometers previously used and should be a valuable aid to producers in determining when to draw off sirup from the evaporators.

Plant Sources of Cortisone Precursors

In 1949 the first reports of the dramatic anti-arthritic action of cortisone were made public. It was soon realized that only plant sources could provide sufficient quantities of cortisone precursors to meet the enormous demand for the new drug. The isolation, from plants, of compounds of biological interest is a field in which the Bureau has unique facilities and experienced personnel; and so, in late 1949 the Eastern Laboratory was directed to make preliminary plans and initiate research on the isolation of steroids from plants. (Cortisone is at present made from a steroid in cattle bile.)

At about the same time it was learned that the Bureau of Plant Industry, Soils and Agricultural Engineering and the National

Institutes of Health were also vitally interested in the cortisone problem. Representatives of the three organizations conferred, and agreed to combine their experience and facilities in attacking the problem. The Bureau of Plant Industry, Soils and Agricultural Engineering would provide plant specimens from all over the world, and would work on agronomic aspects of the problem; the Bureau of Agricultural and Industrial Chemistry at its Eastern Regional Research Laboratory would screen plant specimens for cortisone precursors and extract promising species on a large scale; and the National Institutes of Health, at its National Institute of Arthritis and Metabolic Diseases, would synthesize cortisone from suitable precursors furnished by the Eastern Regional Research Laboratory, and would also work on any physiological aspects of the problem.

The Eastern Regional Research Laboratory currently examines 35-50 samples weekly; as of March 31, 1951, 600 samples had been screened, principally of the order Liliflorae. Since the project started, the screening process has been speeded up by two new technics: (1) a simple hemolytic test which indicates whether or not the desired substances are present in the plant, and if so, (2) an infrared assay to show if the material has the proper chemical structure, i.e., whether it is in the group of steroids known as sapogenins.

It was decided to hunt for a sapogenin with an oxygen atom attached at the 11 or 12 position, for such compounds would be more easily converted to cortisone, in which an oxygen atom at position 11 is a key grouping.

Although chemists have succeeded in introducing an 11-oxygen in "naked" steroids (those with no oxygen at 11 or 12), a suitable 11 or 12 oxygenated sapogenin would still be an excellent precursor. It is estimated that a plant specimen containing only 1% of such a sapogenin would be equivalent to a plant containing several times this amount of a "naked" steroid so far as the final yield of cortisone is concerned. With this background, the following results may be assessed:

(1) Sapogenins with an 11-oxygen. No such compounds have yet been found.

(2) Sapogenins with a 12-oxygen. Manogenin and kammogenin have been found in low yields (0.1-0.2% M.F.B.) in leaves of Yucca gloriosa and Y. filamentosa from North Carolina. Hecogenin has been found in good yield (0.5%) in leaves of four Agave species from New Mexico and Arizona. In addition, there have been found another twelve Agave species with sapogenin contents varying from 0.5-1.0%, from Mexico, particularly lower California.

(3) Steroids lacking a 12-oxygen. Diosgenin has been found in tubers of some six Dioscorea samples from Mexico in yields ranging from 1-3% M.F.B.

Sarsasapogenin has been found in 1.5% yield in the leaves of Yucca thornberi from Arizona.

Many species have yielded low quantities of 12-keto or non-oxygenated sapogenins. It is believed that for commercial success a species should contain at least 0.5% of a 12-keto or several times that much of a non-oxygenated sapogenin. From this it is apparent that the first year survey has met with at least partial success.

Toward the end of the year the second phase of the investigation was reached, namely, the processing of large quantities of promising plant materials so that sufficient sapogenin could be obtained for synthetic work by the National Institutes of Health. Accordingly, 500 pounds of Agave expansa has been obtained and is being processed.

POULTRY, DAIRY AND ANIMAL PRODUCTS UTILIZATION INVESTIGATIONS

Milk Products Utilization Investigations

Protein Investigations

Development of Casein Bristle Fiber

Casein bristle fibers which were treated with phenol-formaldehyde resin to improve the resistance to water have shown considerable improvement in wet strength. By systematic study of reaction conditions, the wet strength has been increased from 0.2 to 0.8 gram per denier in bristle soaked in water at 25°C. for one-half hour. The improvement in wet strength appears to be due to a partial waterproofing rather than a cross-bonding type of reaction. The work is being continued since this is the most successful method of increasing the wet strength yet found.

Curled casein bristle is being furnished to the Mellon Institute for testing as insulation in Arctic sleeping bags. The U. S. Quartermaster Corps is sponsoring this project at the Mellon Institute.

Separation of Caseins

Since casein is the most abundant protein of milk and the most important industrially, continued effort has been directed to the separation of its components by an industrially feasible method. The first method developed depended on the separation of the components from very dilute solutions at low temperatures by variations in pH. This method resulted in making the components available in gram quantities. A second method based on solubility in 50 percent alcohol containing salt resulted in the production of the pure fractions in 100 gram quantities. A third method has now been developed based on the solubility of the components in urea solutions, which will make the pure casein fractions available in pound quantities. If unique uses for pure casein fractions can be found the urea method for separating the fractions will be relatively inexpensive and practical.

Enzymes of Milk

(a) Milk Phosphatase. Purification of milk phosphatase had been handicapped by the adherence of 75 percent of the enzyme to a fatty material as an enzyme-lipid complex of low solubility. The use of

n-butyl alcohol to extract the lipid now permits separation of all the phosphatase in soluble form; subsequent separation from the other whey proteins with the aid of acetone has resulted in a 500-fold increase in enzyme purity.

We have found that milk phosphatase occurs as two types. Type I is adsorbed on Filter-cel and Type II is not so adsorbed. Work on the two types continues, for an understanding of the difference in adsorption properties appears to be basic to an understanding of enzyme chemistry.

(b) Lactoperoxidase. Regular hexagonal plates of crystallized lactoperoxidase have been isolated from solutions of the pure enzyme. Preliminary studies indicate that the purified lactoperoxidase may undergo transformation into the red-colored protein of milk whey. If this observation is confirmed, it will account for another of the milk proteins.

Carbohydrate Investigations

Plasticizers from Lactic Acid

Last year's report described a highly effective plasticizer mixture made by esterification of butyl lactate with adipic acid. This study has been extended to the esterification of capryl lactate with sebacic acid, the process resulting in a mixture of esters of lactic and sebacic acids which may be termed capryl lactate sebacate. The mixed ester is comparable to dioctyl phthalate in its efficiency to plasticize polyvinyl chloride. The fact that this high-boiling plasticizer can be made substantially colorless without resorting to distillation enhances its prospects as a plasticizer for vinyl resins. The Hardesty Chemical Company is evaluating capryl lactate sebacate (mixed esters) as a possible new addition to their line of plasticizers. Of the other lactic acid derivatives evaluated as plasticizers for polyvinyl chloride, the amyl carbonate of dioctyllactamide, the acetate of didecyllactamide, butyl (butyl lactate) maleate, butyl lactate maleate, butyl (butyl lactate) phthalate lactamide, the 2-ethylhexanoate and amyl carbonate of dioctyl lactamide, the acetate of didecyllactamide, and the 2-ethylhexanoate of hydroxyethyllactamide are comparable or superior to DOP in plasticizing efficiency for commercial vinyl chloride resin. Another lactic ester plasticizer developed by the Eastern Regional Research Laboratory, 2-ethylhexyl lactate adipate, is expected to go into plant production on a small scale at Ohio Apex, Inc.

Specialty Rubbers from Lactic Acid

Lactoprene BN, a new elastomer prepared at the Eastern Regional Research Laboratory, is an excellent specialty rubber. This is the second type of synthetic rubber made from acrylic esters which has been developed. (The first, Lactoprene EV, is now in commercial production by B. F. Goodrich and Co. under the name Hycar PA-21). Like its predecessor (Lactoprene EV), the new rubber is resistant to the action of oils, heat, and aging, but in addition, remains flexible at temperatures as low as -25°F. The new rubber, Lactoprene BN, can be made from fermentable carbohydrates through lactic acid; its

principal component is butyl acrylate. The new rubber may be made without close control over processing conditions such as temperature, amount of catalyst and exclusion of air; hence many different manufacturing facilities may be used without adverse effect on the rubber's properties.

For certain uses Lactoprene BN far excels natural rubber and other synthetic rubbers of comparable cost, and it is expected to give longer service life to both military and civilian mechanical equipment. A large automotive manufacturer is currently using Lactoprene BN at critical points in automatic transmissions.

Dairy Waste Disposal

The dilute processing waste from handling fluid milk is a serious source of pollution in many streams. Moreover, lactose is readily converted by bacteria to lactic acid which inhibits further microbiological action. Milk wastes are therefore difficult to treat in conventional sewage treatment plants. A study of the aerobic oxidation of these wastes at the Eastern Regional Research Laboratory has given new knowledge of what happens during an aeration treatment. Two possible new processes have been proposed to the industry on the basis of these results.

The essential finding was that the organic pollutants are assimilated by the microorganisms naturally present with extreme rapidity. The soluble proteins and sugar are thereby transformed to insoluble microbial cells. Two alternative methods of utilizing this synthesis are being considered. The first would require centrifugation or filtration to remove the microorganisms followed by discharge of the innocuous liquid effluent to the stream. A second partial treatment process has been proposed for use when the effluent is to be discharged to a municipal sewage treatment plant or to a stream which is not of the highest quality. It consists of aeration for 8 - 16 hours, followed by discharge of the whole effluent, containing the microbial cells, to the stream. The pollutional load is reduced thereby about 75 percent. This simple daily fill-and-draw operation should be inexpensive to install and operate. Test of it in an operating dairy plant will be made during the coming year if satisfactory cooperation can be arranged.

Production of Amino Acids from Milk Proteins

Since casein is a low-cost protein of high purity, it is an attractive potential source of amino acids which are at present scarce and expensive. Casein can readily be broken down to its constituent amino acids, but separating these acids is extremely difficult. It was found that the occurrence of undesirable side reactions prevents the separation by distillation of the amino acid esters. Attention was next directed to another series of derivatives, the acetyl amino acid esters, and these are more readily separated by distillation. The steps are: hydrolysis of the protein, acetylation, esterification, separation by distillation, hydrolysis of the derivative and crystallization of the amino acids. So far, leucine, isoleucine and threonine have been recovered. The results indicate

stability of the derivatives during distillation, a factor required for the successful industrial use of such a separation.

Animal Fats and Oils and Special Products Utilization Investigations

Synthetic Detergents from Animal Fats

Synthetic detergents not prepared from domestic fats are at present replacing soaps in amounts that would require approximately 350,000,000 pounds of fat for their manufacture. Synthetic detergents derived from animal fats are being manufactured and sold, but as yet in relatively small volume. The problem of suitable competitive detergents prepared from animal fats is being intensively investigated by the Eastern Regional Research Laboratory. Studies being made include preparation and synthesis, washing tests, proper methods of compounding and "building", and other pertinent evaluation data.

Washing tests have demonstrated two materials which can be made from animal fats that are superior to many detergents made from coconut oil or other raw materials. These two are disodium alpha-sulfopalmitate (from palmitic acid) and sodium oleyl sulfate (from oleyl alcohol). Washing tests gave the following order of decreasing excellence: disodium alpha-sulfopalmitate, sodium oleyl sulfate, sodium lauryl sulfate, Tide, Felso, Vel, Surf, Dreft, Igepon T, All. In soft water, sodium oleyl sulfate excelled disodium alpha-sulfopalmitate. A combination of the two, in equal parts, was better in hard water than either one alone. If additional work shows that "building" this synergistic mixture with sulfates, carbonates, borates, phosphates or silicates is possible, the final product may be a highly effective, inexpensive detergent.

Use of Soaps in the Synthesis of Plasticizers

Because of the need for large quantities of plasticizers for the plastics industry, some 28 esters have been synthesized from fatty acids obtained from animal fats for evaluation as plasticizers.

High boiling derivatives of the higher fatty acids are always of interest as plasticizers for rigid plastics and elastomers. Whenever they are compatible with a given plastic, they usually have an advantage in improving water resistance of the product as well as the low temperature mechanical properties. Many compounds in this classification have been found to be compatible with ethyl cellulose and polystyrene, comparatively few with vinyl chloride-acetate, and practically none with cellulose acetate.

Preliminary evaluation conducted on a number of these fatty acid derivatives, including myristates, palmitates, stearates, and oleates, confirmed the above compatibility picture. None of the compounds were compatible with cellulose acetate. Likewise, none were compatible with polyvinyl chloride acetate in sufficient concentration to form a flexible sheet. All, however, were compatible with ethyl cellulose by the hot solvency test.

Of the homologs tested as plasticizers for ethyl cellulose, most were as good or better than the control, n-butyl stearate, in lowering hardness and flow temperature. Both the methyl lactate oleate, and the methyl glycolate oleate appeared to be better than the corresponding stearates, palmitates, or myristates in producing clear, dimensionally stable molded specimens. The methyl derivatives appeared to exhibit slightly lower flow temperatures than either the ethyl or butyl derivatives.

Fat Derivatives of Industrial Importance

Investigations on the use of vinyl esters and ethers of fatty acids for use in modifying the properties of the vinyl-type plastics have been continued with encouraging results.

A series of copolymers of vinyl palmitate and vinyl acetate, covering a wide range of monomer composition, has been prepared. The copolymers show considerable change in properties over the polymerized product from each monomer. Whereas polyvinyl acetate is hard and brittle, the copolymers show increasing flexibility and softness as the percentage of vinyl palmitate in the monomer mixture is increased, and ultimately a tacky product is obtained.

The vinyl palmitate-vinyl acetate copolymers are of interest as lubricating oil additives. It was found that the viscosity index improvement at 5% concentration of additive was significantly increased as the vinyl acetate content of a copolymer was increased. Since polyvinyl acetate is insoluble in lubricating oils, the graded, increased improvement was ascribed to the poorer solubility of the copolymers as the vinyl acetate content was increased.

Preliminary results on several copolymers of vinyl acetate and vinyl palmitate indicate that a copolymer of the proper composition may have utility as a pressure sensitive adhesive for polyethylene sheeting. Since this is an unusual property it is anticipated that the vinyl ester monomers of the fatty acids should have potentialities in the adhesive and printing ink fields. It is known that a need exists for a good adhesive and ink for polyethylene sheeting, a material used in large quantities in the packaging industry.

Utilization of Wool Grease and Other Wool Byproducts

In a continuation of the work reported last year, on the development of a scourable sheep-branding paint, 20 gallons of the fluid fraction of wool grease was prepared. This provided the necessary material for final range tests of the scourable sheep branding fluid developed by the Production and Marketing Administration, preparatory to arrangement for commercial production.

Fundamental work on wool grease was continued by studies of methods to separate the constituents. Attempts to fractionate wool grease by selective precipitation with urea were unsuccessful, as no urea adducts were formed. When the wax was saponified, however, and the alcohol fraction treated with urea, a well-defined adduct was formed and separated. Not all of the alcohols reacted, however, a

result which suggests the presence of an unknown class of alcohols. The fraction left after removal of the urea-adduct-forming compounds can be further fractionated to yield a cholesterol-rich fraction from which it is hoped pure cholesterol can be recovered in good yield.

NORTHERN REGION

Northern Regional Research Laboratory
R. T. Milner, Director

CEREAL AND FORAGE CROP UTILIZATION INVESTIGATIONS

Composition of Commercial Corn Hybrids

Corn breeders need to know whether the oil or protein content of commercial hybrid corn varieties is affected by high or low yields. Although oil and protein are present in corn in much lower proportions than starch, their industrial value per pound is considerably greater.

In collaboration with the Illinois Agricultural Experiment Station, the Laboratory has determined the chemical composition of some 300 varieties of hybrid corn for each of the past 3 years, grown at different locations in the state. It was found that varieties high in oil or protein one year are high in other years. Actual content of those constituents varied markedly, however, with the season and with field fertility. There was no correlation between yield and oil or yield and protein. Thus corn breeders can proceed with developing varieties still higher in oil or protein content without sacrificing yield.

Protein Content of Corn Not Significantly Affected by Pollen Parent

In studies on the composition of corn hybrids grown in experimental plots, there has been some question about the effect of pollen from other varieties upon the chemical composition of the corn analyzed.

To determine the immediate effect of the pollen parent upon the protein content of corn, a cooperative experiment was carried out with the Illinois Agricultural Experiment Station. High- and low-protein strains of corn were pollinated with a mixture of pollen from high-protein and low-protein strains which had different-colored endosperms. Thus, kernels grown on the same ear were obtained from different pollen and the parentage was indicated by kernel color. Analysis of 200 pairs of samples showed that the pollen source had little current effect on the protein content of the grain. Thus, protein content of corn grown in a plot adjacent to other varieties is representative of the variety planted. Likewise, protein content of field-grown corn can be correlated with the variety and is relatively unaffected by cross-pollination.

Composition of Corn as Affected by Maturity and Drying Conditions

In recent years, artificial drying at elevated temperatures has been used to reduce the moisture of high-moisture corn to safe storage levels. High-moisture corn may result from frost damage to immature corn, unfavorable weather conditions during harvesting, or because of the common tendency to use mechanical harvestors before field drying is complete. Industrial processors have generally objected to the processing characteristics of artificially dried corn. A cooperative project with the Illinois Agricultural Experiment Station is in progress to answer questions which have arisen concerning the composition (chemical and nutritional) of corn as affected by maturity at harvesting and conditions of drying.

Results have been obtained on samples of crop year 1949. It was found that protein, sugar, ash, and the vitamins niacin, pantothenic acid, biotin, and riboflavin decreased as the corn matured, whereas starch, oil, and the vitamin pyridoxin increased. Sugar, which is present in relatively large amounts in corn containing more than 50 percent moisture, appears to be largely converted to starch during drying at room temperature, but to a lesser extent at elevated temperatures. Compared to drying at room temperature, artificial drying of high-moisture corn at temperatures above 130° F. minimized loss of niacin, but increased the loss of pantothenic acid and pyridoxin. Most significant was the finding that the composition of corn harvested with less than 40 percent moisture was little affected by the temperature of drying.

Flour Milling Industry Aided by New Data on Wheat

The fact that some wheats do not mill as well as others is well known to flour millers. Some Pacific Northwest wheats, such as Rex or Brevor varieties, slow up flour production when put through the mill. An understanding is needed of the structural and chemical factors within the wheat kernel which determine whether the variety has good or poor milling quality. Early detection of a poor milling variety by microscopic or chemical means would enable the wheat breeder to discard it before much time and effort are expended. Present evaluations of milling quality require sufficient wheat to conduct actual milling trials. Furthermore, a knowledge of factors which affect milling quality may allow the development of a treatment to improve the milling character of a poor milling wheat which, because of its good agronomic character, might be popular with farmers.

A number of wheat varieties, ranging from poor to excellent in milling behavior, have been studied microscopically. Differences are sought which parallel differences in milling qualities.

Work recently completed on seven varieties of white wheat grown in the Pacific Northwest shows that bran thickness, previously thought to affect the ease of milling and the amount of flour obtained, has no relationship to these factors. Likewise, thickness of each of the different layers which make up the bran shows no relation to the milling behavior. The results found thus far indicate that factors responsible for milling quality must reside within the inner part of the kernel. Studies are in progress to find out which of the structural and chemical factors inside the kernel determine the milling quality.

Sugar Content of Sorghum Fodder

The increased use of sorghum fodder as a winter feed for range cattle has focused attention on its keeping quality, a factor which appears to be affected by the type of soluble sugars present. There is also interest in the production of sucrose from sweet sorghums by beet sugar factories during their off-seasons. In cooperation with the Ft. Hays (Kansas) Experiment Station, 29 commercial varieties and new strains of fodder sorghums were analyzed. The amount of reducing sugar ranged from 1.9 to 26.9 percent and the total sugar from 5.2 to 53.9 percent on a dry basis. Reducing sugar constituted from 18.5 to 78.6 percent of the total sugar. Although the keeping quality of sorghum fodder for feed is favored by a low sucrose content, production of sugar from sorghum quite obviously requires a high sucrose content. The variation in sugar composition of the varieties analyzed suggests that suitable varieties might be developed for either purpose.

Transparent Wrapping Material Obtained Experimentally from Starch

The supply of transparent films for packaging of foods and other selected items for retail trade has steadily fallen behind demand.

A method has been developed for the laboratory preparation of a new type of self-supporting, transparent film from amylose, a constituent of the common starches. The dry tensile strength, flexibility, tear resistance, and bursting strength of the high-quality amylose film are comparable to those of present commercial packaging and wrapping films. An outstanding characteristic of amylose film is its digestibility by the body. This special property suggests uses in the food packaging and pharmaceutical industries, and cooperative studies with food processors on adaptation of film to packaging of food are in progress.

Amylose film can be plasticized, and other modifiers or additives can be incorporated in the film to adapt it to special uses. Colorful films for decorative purposes can be obtained by addition of dyes. Coating of objects with amylose film can also be accomplished by spraying or dip-coating techniques.

Industrial application of amylose films can be expected if economical methods are found for obtaining amylose, either by improvement of procedures for fractionation of cereal starches, or by obtaining starches of high amylose content from special plant varieties other than those now grown.

Improved Acetates of Starch and Other Carbohydrates Developed

A modification of a previously known method for acetylating carbohydrates has been developed for starch, amylose, amylopectin, and dextran, a versatile polymer of glucose produced by certain bacteria. This new procedure overcomes undesirable breakdown of products during the reaction and produces good yields of acetate derivatives with greatly improved properties. The practical and useful solubility characteristics of the new acetate derivatives have not been obtained heretofore. Acetates of the starch fractions, amylose and amylopectin, made by the new procedure, are soluble not only in halogenated hydrocarbons but also in acetone, a preferred and low-cost solvent. Dextran acetates are completely soluble in halogenated solvents.

In contrast to cellulose acetate, important in the manufacture of acetate rayon, non-flammable film, and plastics, the acetates of starch, starch fractions, and dextran have had restricted usefulness in industry and research because of inadequate methods of preparation, degradation of the product during preparation, and insolubility of the acetates in low-cost, commercially available solvents.

The new experimental method of carbohydrate acetylation, which is uniquely effective because of the use of the commercially obtainable chemical, formamide, makes the prospects more favorable for developing uses for acetates of starch, amylose, amylopectin, and dextran similar to those of cellulose acetate.

Dextran as a Blood Plasma Substitute

Within the last few years accelerating interest has been manifested in the U.S. in dextran, a gum-like polymer obtained by action of certain organisms on sucrose, as a source of material suitable for maintaining circulatory volume when blood or blood plasma either were not available or were in insufficient supply. It is not foreseeable that, under present methods of collection and storage, there could be adequate amounts of whole blood or blood plasma stockpiled to meet the heavy requirements of mass casualties from shock, burn, and hemorrhage. Some replacement or substitute material for blood and blood plasma is a necessity to insure treatment in the event of an all-out modern war.

Much fundamental biological, chemical, and engineering data are needed to put the preparation and use of dextran on an intelligent and scientific basis. For the first time, a comprehensive survey is being made of the types of dextran produced by

a large variety of bacteria. The primary objective of the survey is to find microorganisms which can produce a dextran having especially favorable characteristics. Approximately 200 dextran preparations have been isolated at the Northern Laboratory from dextrans obtained from the fermentation reactions of about 70 different organisms. Evidence collected thus far in the survey is forming the basis for selection of dextrans of widely different chemical structure. The object is to determine the most satisfactory raw material for the production of a fraction of partially depolymerized dextran having a molecular structure and molecular size suitable for the preparation of a blood plasma extender solution. Additional strains of microorganisms in this Laboratory's Culture Collection which are believed to be potential dextran producers are being screened.

The dextrans are differentiated and characterized on the basis of solubility, stringiness or elasticity of the gum, type of turbidity or fluorescence of aqueous solutions, and degree of branching as indicated by oxidation with sodium metaperiodate. Dextrans have been obtained which differ widely in these characteristics and also in the combination of the characteristics shown. To determine whether different dextran types can be obtained from one strain of organism by variation in culture conditions, dextran from some organisms has been produced under as many as nine different conditions of medium and aeration. Tentative conclusion is that the dextran is characteristic of the organism producing it but can be changed by the variation of culturing conditions.

In the production of dextran, it has been found possible to use low-cost substrate materials in the whole culture method employed in the laboratory, and also to produce dextran in pilot-plant equipment. Also, a means has been developed for producing cell-free dextran-synthesizing enzymes, an important step where use of dextran as a blood plasma extender requires minimal contamination by bacterial particles and serologically active proteins.

Initial studies have been made and methods developed for the partial depolymerization of bacterial dextran by acid hydrolysis into a molecular size suitable for intravenous injection. Preliminary investigations also have been made on the partial depolymerization by physical means in an attempt to discover improved methods for obtaining preferred molecular structure for clinical study.

An improved method for the determination of dextran by the anthrone color reaction has been developed to follow more rapidly and accurately those phases of the dextran project concerning fractionation, purification, and molecular weight determinations. Molecular weight is an important factor in determining the suitability of a dextran as a plasma supplement, for it is directly related to the colloid osmotic pressure and the retention of the dextran in the circulatory system. Although there is no clinical evidence concerning the effect of molecular size and shape as independent

of molecular weight, these properties may also be important. In the present investigation of dextrans produced by different microorganisms and of fractions from the degradation of dextrans, these molecular properties have been measured by light-scattering, osmotic pressure, and viscometric methods.

Analysis of Complex Sugar Mixtures Now Possible

As a result of the Laboratory's work on the development and use of new paper chromatography techniques, both qualitative and quantitative analyses of complex mixtures of sugars now can be carried out rapidly and accurately. A few drops of the sugar solution suffices for an analysis. The chemical treatment of carbohydrates often results in a complex mixture of sugars, the analysis of which is very time consuming or even impossible. Many such analytical difficulties have now been overcome with the new chromatographic techniques.

These advances are particularly significant and valuable in the study of such problems as the conversion of corn starch to corn sirup and corn sugar, the breakdown of starch by enzymes for fermentation, the preparation and purification of new sugars, the determination of the nature of the carbohydrates in plant materials and products of microbiological origin, and the breakdown of high molecular weight dextrans to produce fractions suitable for blood plasma extenders. These new developments in paper chromatography of carbohydrates already have proven indispensable in several problems of the Laboratory.

Fermentation Process Developed for Making Itaconic Acid

Pilot-plant studies on a process for making itaconic acid by a fermentation method, developed in the laboratory, have established preliminary costs for this product which has potential use in plastics for industrial and military purposes. According to the preliminary estimate, based on current price levels, itaconic acid may be produced and recovered by the process for 27 cents per pound in a plant producing 3 million pounds annually. At this production cost, itaconic acid could compete economically with a variety of dibasic acids, such as maleic and fumeric. Esters of itaconic acid could compete economically with methacrylate esters, with which they would most probably be compounded for the production of plastics.

Although itaconic acid is not an important product in commerce at present, its properties are such that, if available at a reasonable cost, it could be used in large quantities in producing flexible, strong, transparent plastics as are needed for fabricating noses, domes, turrets, and windows of military aircraft and for optical instruments for military equipment. Excellent transparent plastics of the thermoplastic type can be made by the copolymerization of itaconic acid esters with

methacrylate esters, vinyl chloride, or acrylonitrile. The resulting plastics possess higher flexural and impact strengths than those made without itaconic esters.

New Fermentation Method for Production of Vitamin B_{12}

An increased yield in the production of the important animal food factor, vitamin B_{12}, by fermentation has been obtained with a new microorganism. Noteworthy, also, is the capacity of this organism (Streptomyces olivaceus) to produce an unidentified antibiotic substance simultaneously with vitamin B_{12}. Feeding trials with poultry showed the preparation to supply the vitamin B_{12} needs plus an additional growth stimulation believed to be due to the antibiotic substance. Under a research contract, the Michigan Agricultural Experiment Station will conduct further feed trials on these materials.

A feature of this deep-tank fermentation is a relatively simple and inexpensive medium composed of mineral salts, carbohydrate, and a material supplying nitrogen, such as soybean, cottonseed, or peanut oil meal, distillers' solubles, or mycelium from the penicillin fermentation.

Although vitamin B_{12} is recovered as a byproduct of the fermentation production of such antibiotics as aureomycin and streptomycin, there is still need for its large-scale production to supply the pharmaceutical and animal feed needs. Culture medium and conditions developed in the laboratory were found to give good yields in trials with large-scale, conventional equipment. At least two companies were immediately successful in the use of the new process for quantity production of vitamin B_{12}, and other companies are seriously interested in the process.

Zymonic Acid, A New Product from Yeasts

The remarkable synthetic powers of the molds and bacteria have been amply demonstrated throughout the years by the isolation of scores of metabolic products such as acids, alcohols, ketones, antibiotics, and vitamins. The abilities of the yeasts in this regard, however, have received relatively scant consideration except for the well-studied alcohol fermentation. As part of a search for industrially useful fermentation products, an effort has been made to fill partially this gap in our knowledge by surveying in aerated culture about 120 species of yeast from 24 different genera. This study has resulted in the isolation of a new acid to which the name of "Zymonic Acid" has been assigned. Chemically it is known as 3,5-diketo,4-methyl, tetrahydrofuroic acid. It is produced by three different species of yeast from widely different genera which suggests that the acid may be of fundamental importance in the breakdown of sugars by yeasts. Zymonic acid belongs to the class of unstable compounds known as the β-keto acids which figure prominently as postulated intermediates

in the degradation of sugars by other microorganisms, plants, and animals. Hence, the actual isolation of such an acid may help in establishing with more certainty the mechanism whereby living things derive their energy from sugars. Also indicated is the possibility that more new chemicals may yet be discovered among the products of metabolism of yeasts and other microorganisms.

Alcohol in Motor Fuel Has No Adverse Effect on Premium-Grade Lubricating Oils

Studies on the use of alcohol as a motor fuel in internal combustion engines have taken into account the possibility that alcohol, either blended with the gasoline fuel or injected as an alcohol-water mixture into the intake manifold of an engine operating on gasoline, might have a detrimental effect on "additives" (antioxidants, corrosion inhibitors) in the premium-type lubricating oils recommended for use in most passenger cars.

Some outstanding results were obtained in tests made under research contract by the Armour Research Foundation which showed that under ordinary driving conditions, alcohol-water injection reduced the loss of weight in the test bearings, one of the several criteria used in judging the performance of the lubricating oil used.

During some of the test runs, it was observed that sludge deposits showed a tendency to increase with the use of alcohol injection. No severe sludging occurred, however, in any instance.

Within limits of the investigation, it was shown that injection does not interfere with the action of lubricating oil additives but actually may improve their effectiveness in some respects.

OILSEED UTILIZATION INVESTIGATIONS

New Microanalytical Methods

Two new microanalytical methods have been developed, one for the determination of the saponification equivalent of samples in the range of 0.05 milliequivalent, and the other for the determination of nitrogen in nitrile compounds. The first method permits the analysis of small samples, such as are available from the Craig countercurrent distribution apparatus, and has the further advantage that it is applicable to volatile esters which could not be analyzed by previous micro methods.

In developing the second method, it was demonstrated that the ordinary Kjeldahl procedure for nitrogen could be applied to nitriles without the preliminary reducing treatment involved in accepted methods. Examination of the literature revealed that the original use of the reducing treatment was on a nitrile which also contained a nitroso group. It would seem that the reduction was intended for the nitroso group and that its application to nitriles as a class was carried into later publications without critical consideration. Elimination of the reducing pretreatment decreased

the time and work required for the determination of nitrile nitrogen in cyano derivatives of amino acids prepared in this Laboratory.

Composition of Soybean Phosphatides

In studies on the liquid-liquid fractionation of soybean phosphatides, it was found that alcoholic washing of the phosphatides reduces their sugar content substantially. The residual sugar appears to be chemically bound to the hexane-soluble phosphoinositide. Studies on the use of the sugar-free phosphatides as an inactivator of metals during the deodorization of soybean oil showed that the discoloration of the oil caused by the phosphatides was not reduced. Cadmium-precipitated lecithin gave only a slight discoloration, indicating that the phosphoinositide or cephalin fractions were responsible for the darkening. It appears likely that the browning and discoloration of phosphatides is an aldehyde-amine reaction and that sugars may not be involved.

Purified fractions of soybean and corn phosphatides were evaluated for use in the intravenous feeding of oil emulsions by an ethical pharmaceutical company. They reported that all fractions when incubated for 1 month developed toxicity to mice when injected intravenously. This observation of toxicity on incubation has been common to all previous samples of soybean "lecithin" or fractions thereof. It appears that all fractions of soybean "lecithin" will develop toxicity for intravenous injections.

The "free" sugars present in soybean "lecithin" have been identified as sucrose, raffinose, and stachyose in relative amounts of 7, 1, and 6. Upon removal of the sugars by alcoholic washing, phosphoinositides which are combined with sugars are obtained by solvent fractionation. These sugar-containing phosphatides are concentrated in the hexane-soluble fraction. Galactose and arabinose are the sugars combined with this fraction.

Soybean Lecithin Chemically Modified

Chemical modification of commercial soybean lecithin has been accomplished at the Northern Laboratory. Reaction of lecithin with isocyanates has given products varying in physical form from viscous liquids through soft and hard waxes to hard, brittle, resin-like solids, depending on the type and amount of isocyanate employed.

A waxy, semi-brittle product was prepared on a pilot-plant scale by reacting lecithin with phenyl isocyanate. In contrast to the unmodified lecithin, it formed with comparative ease water suspensions which have reduced surface tension and improved stability to microbial action. Upon request, this product has been supplied for evaluation to potential users, such as manufacturers of printing inks and floor waxes.

Dilinoleic Acid--A New Chemical from Vegetable Oils Now Produced Commercially

Dilinoleic acid ("dimer acid"), a dibasic, unsaturated fat acid derived from soybean and other vegetable oils, is now in commercial production by a new process. Manufacture of this new chemical, which has a potentially widespread industrial use, was stimulated by pioneering research on this material at the Northern Laboratory and by continued cooperative research which has been carried on between this Laboratory and chemical industries.

Dimer acid has been studied extensively at the Northern Laboratory for potential use as an industrial chemical. In a series of technical papers on the utilization and properties of dimer acid, issued by the Laboratory during 1942 to 1949, information was given on how industry could use the acid for upgrading drying oils and for manufacturing rubber replacements, polyamide resins, viscosity-index improvers, and synthetic rubbers. The polyamide resin "Norelac," which was developed at the Northern Laboratory from soybean oil, was discussed in the 1945 and 1948 reports.

During World War II, industrial companies undertook production of rubber replacements and polyamide resins from dimer acid, and commercial utilization of these products was achieved. Need for rubber replacements became less urgent with the success of the synthetic rubber program, but commercial manufacture of polyamide resins has continued because of the demand for this material as a moisture-proofing and heat-sealing agent, particularly for glassine wrappers for candy bars and other food products. Recently a more extended use of dimer acids in drying oils and protective coatings is indicated because of the reduced quantity of tung oil available for domestic use.

In consequence of the increasing interest in dimer acid, so largely stimulated by the work of the Northern Laboratory, one company investigated better methods of producing the acid. A successful process was developed and dimer acid is now available in commercial quantities.

Modification of Soybean Oil with Mono- and Polyfunctional Organic Chlorides for Lubricants

Pure compounds are being prepared to study the viscosity characteristics of the 18 carbon acid and how it may be modified to obtain high viscosity indices as well as low pour points.

It was found that alkali dehydrochlorination of methoxychlorostearates removed 90-98 percent of the chlorine to produce ketostearic acid and a product which had the properties of a methoxyoctadecenoic acid. The yields of ketostearic acid from oleic acid were 61 or more percent with remainder presumably

being a mixture of methoxyoctadecenoic acids. From elaidic acid, lower yields of ketostearic acid were obtained and presumably more octadecenoic acid. Since modification of the 18 carbon chain with methoxy or other alkoxy group appears to be important to obtain good low-temperature characteristics for lubricants, these observations on oleic and elaidic may have considerable importance when further data become available.

Linolenic Acid is a Cause of Undesirable Flavors in Soybean Oil

Studies at the Northern Laboratory have shown that linolenic acid is a flavor instable component of soybean oil. It had been demonstrated experimentally that the flavors peculiar to aged soybean oil could be imparted to the otherwise flavor-stable cottonseed oil by interesterifying or introducing linolenic acid into cottonseed oil.

Further confirmation of the major part which linolenic acid plays in causing the undesirable flavors has now been obtained. Some of the volatile flavor principles isolated and identified by the University of Pittsburgh (contract research) have been found to be formed by storing linolenic acid esters in the presence of oxygen. Moreover, studies of the oxidation of linolenic acid esters have demonstrated that it oxidizes differently from the esters of cottonseed oil. Cleavage of the linolenic acid molecules to give volatile flavor principles occurs at the very early stages of storage. This stands in contrast to the much later occurrence of these chemical reactions in esters of flavor-stable oils. The apparent difference in reaction mechanism for linolenic acid esters may account for the difference in flavor stability of soybean and cottonseed oils. This additional proof of the importance of linolenic acid to flavor stability will assist in devising means to utilize more soybean oil and its products in foods.

Corn Acid Stabilizes the Flavor of Soybean Oil

Phytic acid is the most recent and promising compound discovered at the Northern Laboratory for stabilizing soybean oil. Phytin, the calcium and magnesium salt of phytic acid, is present in many of our foods, particularly the cereal grains. It is commercially available from the corn wet milling industry where it occurs as a component of the steep water. Like citric acid (discussed in previous reports) and sorbitol, both of which have been advocated by this Laboratory and widely accepted by the soybean oil industry, phytic acid stabilizes soybean oil by virtue of its ability to inactivate naturally occurring and contaminating trace metals. The metals, principally iron and copper, when uncombined, start or accelerate the oxidative deterioration of the oil and speed the development of undesirable flavors and odors. Phytic acid, however, has a distinct advantage over citric acid, sorbitol, and related compounds. When this acid is added at the beginning of the deodorization step in refining soybean oil, its capacity to counteract the injurious effect of metals present in the oil is evident, not only during deodorization, but also

throughout the subsequent storage life of the oil. Pilot-plant studies and semi-commercial trials indicate that phytic acid added to the deodorizer at a rate of 1.5 to 3.0 ounces per ton of oil gives deodorized oils of initially high flavor quality and of improved stability during storage. If thorough testing proves that phytic acid as a stabilizer for soybean oil has no harmful effect on the consumer, it can also be used to advantage in improving the quality of hydrogenated shortenings made from the oil and used in baked goods, candies, and other foods. The "shelf life" or storage life of such products might be increased by using the improved soybean oil products, and these foods, therefore, would "keep" better in the grocery and in the pantry. Also, military rations might be improved by making it possible to include more of the high energy fats and oils that soldiers want and need but that are likely to develop undesirable flavors unless the oil is stabilized.

Soy Flour Improved for Foreign Use

The use of soyflour in bread has been urged for European countries during the past several years because of the need for more protein in their diets. Its use there, however, presents problems different from those in the United States. Europeans use long-extraction wheat flours of rather low protein content and much of their bread is made without sugar or shortening. In Greece, for example, flour of 90-percent extraction is used and never any sugar or shortening. Attempts by Greek bakeries to use soyflour in bread were so unsatisfactory that in one year less than 10 percent of their allotment of soyflour was used.

A series of experiments conducted at the Northern Laboratory on Greek-type bread showed that oxidizing agents such as potassium bromate--an approved bread ingredient--were of major importance when fortifying high extraction flours with soyflour. When 5 percent of soyflour was used without oxidizing agents, the bread was very inferior. The volume of the loaf, which is normally low compared with that of bread in the United States, was reduced to two-thirds of its original volume; thus the bread became unappetizing even to people accustomed to heavy bread. It was found that on addition of the correct amount of potassium bromate, the loaf volume became normal and other undesirable characteristics disappeared. However, it was evident that because of the crude working conditions and the small size of the Greek bakeries, the bakers would be unable by themselves to use the oxidizing agents. Hence, it was suggested to the Economic Cooperation Administration that the correct amount of potassium bromate be added to the soyflour at the time of its manufacture. Through the cooperation of the soyflour industry of the United States, a quantity of soyflour was manufactured and the oxidizing agent was added at the specified level. One thousand tons of this bromated soyflour were purchased by ECA and shipped to Greece for trial there.

Development on a Pilot-Plant Scale of a Solvent Extraction Process for the Recovery of Oil and Meal from Safflower Seed

Experimental tests have been made in the pilot-plant equipment on the extraction of safflower presscake. A quantity of presscake produced in a small screw press at the University of Nebraska and containing about 8.6 percent of oil on a moisture-free basis was obtained for the experimental work. A series of experimental runs was made in the pilot-plant equipment in which the solvent was reduced from an initial value of 1.0 down to 0.45 parts per part of presscake on a moisture-free basis. In view of the excellent results obtained, a test was made with presscake which was ground in an attrition mill but not flaked. The extraction progressed satisfactorily, the average residual oil content of the extracted cake was low, and the miscella contained only a small quantity of "fines."

Studies were made on the development of a suitable method for the preparation of whole safflower seed to be used in the solvent extraction step, and various types of mills were tested. An Abbe mill containing stationary blades and a set of cutting blades mounted on a rotor appeared most promising. The hulls are cleanly cut into smaller fragments, and little oil is exuded during the operation. It is necessary, however, to use sharp knives and to clean the mill regularly. Good results were obtained at a moisture level of 9 to 10 percent, and the cutting action knocks the meat particles free from the hulls. A portion of the hulls can be removed by aspiration, if desired.

SUGAR AND SPECIAL PLANTS UTILIZATION INVESTIGATIONS

Citric Acid Obtained from Cheaper Materials by New Submerged Fermentation Method

The desirability of a satisfactory deep-tank fermentation process to produce citric acid is widely recognized. This important food and beverage acid, produced in quantities amounting to about 35 million pounds per year, is now made by fermentation of beet or a mixture of beet and cane molasses solutions in shallow pans. To date, no submerged process has been reported whereby cheap raw materials can be utilized without expensive preliminary purification to remove the traces of iron and manganese which retard the fermentation.

It was discovered at the Northern Laboratory that addition of methanol to submerged shaker-flask cultures in which cornstarch, finely ground corn, commercial glucose, or blackstrap molasses were being fermented to citric acid, gave increased yields ranging from three- to twelve-fold. The fact that the yields of acid from the various lots of blackstrap molasses used were essentially the same as those obtained from commercial glucose indicates the suitability of the cheaper carbohydrate as raw material. Several commercial companies are planning to try out this methanol procedure.

AGRICULTURAL RESIDUES UTILIZATION INVESTIGATIONS

Fine Papers and Newsprint Made Experimentally from Wheat Straw

Advancing prices of wood pulp and shortage of newsprint have stimulated interest in fine straw pulp. In cooperation with a West Coast paper company and the Forest Products Laboratory, a series of fine papers (book, magazine, writing, and waxing) and newsprint was made containing 20 to 50 percent pulp from either Pacific Northwest or Illinois wheat straws. All of the papers containing straw had better formation, strength and surface characteristics than did the all-wood pulps. The results of these paper mill runs were so successful that the paper company expects to do further work in its own plant with the hope of using straw as a blend to improve certain of its present wood pulps.

In these experimental paper runs, opportunity was also afforded to try out the idea advanced by the Northern Laboratory that newsprint could be manufactured from de-inked waste newsprint and a certain proportion of fine bleached straw pulp. For this purpose unprinted waste newsprint was repulped and mixed with 20 percent bleached straw pulp. The paper proved to be superior to standard newsprint in color, strength, and surface characteristics. It seems practical, therefore, to give consideration to relieving the shortage of newsprint by producing fine straw pulp and blending this with de-inked, repulped waste newsprint.

Process Developed for Making Boxboard from Straw to Replace Wood Veneer in Wire-Bound Shipping Containers

Approximately 1.5 billion square feet of wood veneer is used annually in wire-bound shipping containers. Such containers require only about half as much lumber as the all-wood cases and their use results in a savings not only in lumber but also in freight costs. Uses for wood veneer have been increasing rapidly during the past few years which has resulted in increases in prices and a shorter supply of veneer for the manufacture of shipping containers.

In cooperation with a leading manufacturer of box-making machinery, a process has been discovered for making boxboard from straw. Boxes made from this board, edged with metal, and exhaustively tested in a packaging laboratory, proved equal and in some respects superior to similar boxes made from wood veneer. Industry is considering installation of a mill to make straw boxboard to supplement the supply of increasingly expensive wood veneer boxes. Wire-bound shipping containers represent a potential outlet for about 800,000 tons of straw per year.

Estimates have been made by a prominent engineering company on the costs of constructing a plant to produce 123.5 tons of straw boxboard per day and on the costs of board manufacture. Plant

construction costs were estimated to be $3,875,660; direct daily production costs, $37.40 per ton; and indirect costs, $12.05 per ton. Allowing 10 percent for variables, the total manufacturing cost was estimated at $54.40 per ton. Based on a price in December 1950 of $110 per 1,000 board feet for veneer f.o.b. Southern Mills, it was estimated that the straw boxboard, including metal edging, could be manufactured and sold in competition with veneer at a 26 percent profit before Federal income taxes. Veneer was priced in February 1951 at $120 to $140 per 1,000 board feet.

New Chemicals Made from Corncobs and Other Residues

Furfural, a compound made largely from corncobs, has found its chief uses in refining lubricating oils and as a raw material for nylon production.

Laboratory studies have shown that the simple reaction products of furfural with acetaldehyde and with acetone can be converted by treatment with hydrogen and water into water-soluble trihydroxy compounds resembling glycerine in many of their chemical and physical properties. Treatment of these glycerine-like products with fatty acids yielded a series of new compounds which are glyceride-like esters. These esters have properties which indicate that they may find uses as synthetic lubricants for aircraft instruments, as high-quality plasticizers for vinyl and cellulose acetate films, or as components of hydraulic fluids for automobiles and airplanes. In addition, as coproducts with these trihydroxy compounds, the hydrogenation process as applied to furfural condensation products produces cyclic ether-substituted alcohols which appear to be promising intermediates for the synthesis of new solvents, plasticizers, and lubricants.

By variation and control of hydrogenation conditions, it has been shown possible to convert the three simple condensation products of furfural with acetaldehyde and with acetone into 19 different chemical compounds. Preparation of derivatives and further processing reactions can easily result in the synthesis of hundreds of new chemicals basically derived from agricultural residues via furfural.

Grinding Studies on Corncobs and Nut Shells

The increasing importance of processing corncobs for industrial purposes is shown by the increased demand for cob products by the consuming industries and in the growing list of cob processors. The Laboratory is called upon almost continuously to provide assistance to processors and consumers. As a result of this cooperative action, the quality of the commercial corncob products has been improved with benefit to both the processor and the consumer. Merchandising efforts of some processors have finally resulted in sales of all the products of grinding. Soap manufacturers and compounders and molders of hard rubber products use substantial quantities of corncob meal and flour, heretofore

slow-moving items, at processing plants. Studies on stored-grain protectant dusts have shown that many agricultural residues when properly processed are suitable for this purpose. Corncob flour is receiving considerable attention by one insecticide manufacturer who contemplates a possible demand of 5,000 tons of the flour for the year 1951.

As a result of this Laboratory's activity in this field and the growing demand for ground agricultural residues, principally corncobs, the number of known processors or grinding plants has increased to 19 for corncobs and 5 for shells of nuts and fruit pits. Twelve of the cob plants are processing for industrial purposes, while the remainder are grinding for agricultural applications. Two cob processing plants are reported under construction and two additional cob plants and one nut shell processing plant are being given serious consideration.

Better Utilization of Sugarcane Residue Possible

Bagasse (the residue left after squeezing the juice from sugarcane) and blackstrap molasses are the cane sugar industry's primary byproducts, neither of which is being used to the fullest advantage. Bagasse is comonly burned as fuel in the mill to generate steam, although much of it is burned simply to dispose of it. Several sugar mills in the United States bale bagasse and sell it to manufacturers of insulating board. A few mills convert the bagasse into mulch and chicken litter. Blackstrap molasses has not found a stable market price for years. Although its use for livestock feeding is increasing, its price has been based on the alcohol market.

A new wet mechanical method has been developed for separating fibers completely from the pith as the bagasse comes from the cane mill. Both pith and fiber are then dried. When the pith-free bagasse obtained by the new method is chemically pulped, it yields a superior material for making paper--an especially significant discovery in view of the serious pulp shortage which is developing in the United States. The absorbent pith, mixed with byproduct molasses, will make the molasses a more acceptable feed ingredient. The Laboratory's experiments so far completed have aroused much industrial interest because they indicate that operation of this method might possibly give the sugar mill an additional profit of nearly $1 per ton of cane processed.

BIOLOGICALLY ACTIVE COMPOUNDS DIVISION
Beltsville, Maryland
Thomas D. Fontaine, Head

BASIC INVESTIGATIONS IN THE CHEMISTRY OF AGRICULTURAL PRODUCTS

Tomatidine, a new biologically active compound isolated from tomato plants, has been characterized as a new steroidal secondary amine. A potentially useful sterol, Δ^{16}-allopregnen-3(β)-ol-20-one, has been obtained by the chemical degradation of tomatidine. Thus, tomato plants have become a potentially important domestic source of a starting material which may be useful in the production of sex hormones and cortisone.

Two types of plant-growth regulators have been synthesized - namely, quaternary ammonium carbamates, and 2,4-dichlorophenoxyacetyl derivatives of DL, L, and D amino acids. These and other compounds have been tested for growth regulating activity, in cooperation with the Bureau of Plant Industry, Soils and Agricultural Engineering.

Analogs of the new plant-growth regulator "Ammo 1618" (2-isopropyl-4-dimethylamino-5-methylphenyl 1-piperidinecarboxylate methyl chloride) have been prepared. These compounds have been found to be active against Red Kidney bean, sunflower, tobacco, lettuce, calliopsis, and ageratum. The 2-isopropyl group and the 1-piperidinecarboxylate ester group can be replaced by the tertiary-butyl group and by the dimethyl or diethylcarbamate ester groups, respectively, without an appreciable change in activity.

The activity of the 2,4-dichlorophenoxyacetyl-DL-amino acids varied from 14 to 106 per cent of that of 2,4-dichlorophenoxyacetic acid (2,4-D) when compared on an equimolar basis and expressed in terms of reduction of growth of bean plants. In order to establish the significance of these results, the 2,4-dichlorophenoxyacetyl derivatives of the optically active isomers of ten amino acids (D and L forms) were made and tests are now being conducted to determine the structural specificity of these compounds.

PRODUCTION OF PLANT-GROWTH REGULATING COMPOUNDS FROM AGRICULTURAL SOURCES; RM:a-272

Corn pollen, distillers' solubles, and immature beans have been found to contain plant-growth regulating compounds. Indoleacetic acid has been identified in extracts prepared from each source, but this compound accounts for only a portion of the observed activity of purified fractions. Results obtained thus far show that at least one additional plant-growth regulator is present in each source material. From the standpoint of plant response and chemical and physical tests, these unidentified plant-growth regulators differ from one another in chemical composition and action in the plant.

PRODUCTION OF ANTIBIOTICS FROM AGRICULTURAL SOURCES; RM:a-145

Investigations into the nature of the substances in grapefruit peel responsible for its antifungal activity has resulted in the isolation of four compounds, two of which exhibit antibiotic activity. A large quantity of dried grapefruit peel, which is a commercial by-product of the citrus industry, has been extracted and the compounds obtained from this extract are being identified and evaluated. The active materials are of a steroidal nature.

Levulinic acid has been detected in numerous plant extracts and may account for some of their antifungal activity. In the case of certain molasses samples, which were reported to be of poor quality for citric acid fermentation, a small amount of levulinic acid was isolated. However, it was concluded that factors other than levulinic acid must account for the poor fermentation result.

Scabrin, a new insecticidal compound, which has been isolated from Compositae Heliopsis helianthoides var. scabra by the Bureau of Entomology and Plant Quarantine was found to inhibit the growth of 16 microorganisms. Several synthetic analogs of scabrin were found to be essentially inactive as antibiotics and it is of interest to note that they were inactive as insecticides.

The antibiotics present in sweet potato extracts have been further purified. A chloroform extract of an active concentrate yielded a resinous material which was active against fungi and Mycobacteria. A small amount of active crystalline material has been obtained from this resin and it will be characterized when additional material is isolated.

Attempts have been made to purify and separate the antibiotics present in banana extracts by chromatographic methods. Activated alumina and mixtures of talc and hyflo supercel failed to adsorb any of the antibiotic activity, but when an activated carbon was used the antifungal factor was adsorbed and the antibacterial factor passed through the column. From the banana extract a yellow resin, active against fungi, was obtained. After repeated purification by liquid-liquid extraction and solvent recrystallization procedures a white amorphous material was obtained. This material gave a positive test for a sterol and, upon treatment with acetic anhydride, formed a crystalline sterol acetate.

ALLERGENS RESEARCH DIVISION
Washington, D. C.

Henry Stevens

BASIC INVESTIGATIONS IN IMMUNOCHEMISTRY OF AGRICULTURAL PRODUCTS

SEROLOGICAL STUDY OF MILK PROTEINS — Serological methods were applied to test the homogeneity of crystalline beta-lactoglobulin which had been isolated by electrophoresis. Normal beta-lactoglobulin, formerly thought to be a homogeneous protein, was found to contain at least 2 proteins. One of the two components was isolated as a crystalline protein showing a single electrophoretic boundary over the conventional pH range. This apparently homogeneous protein was found to contain, by serological tests, about 0.04% milk albumin. No milk albumin was detected in the normal beta-lactoglobulin and no other difference in serological specificity was detected between normal beta-lactoglobulin and the crystalline preparation derived from it. During attempts to isolate a homogeneous beta-lactoglobulin at ERRL, a crystalline derivative was prepared from beta-lactoglobulin containing two equivalents of firmly bound dodecyl sulfate. This derivative of beta-lactoglobulin afforded opportunity to study the effect on the antigenic specificity of the protein of the introduction of a foreign polar compound. For comparison, a similar product was prepared from serum albumin, which yielded a second protein likewise containing two equivalents of dodecyl sulfate. These two preparations were compared in serological tests with each other and with their native protein counterparts. The results were: (1) Beta-Lactoglobulin dodecyl sulfate did not cross-react with serum albumin dodecyl sulfate. (2) Beta-Lactoglobulin dodecyl sulfate was qualitatively and quantitatively identical with normal beta-lactoglobulin in Dale tests. (3) Serum-albumin dodecyl sulfate was quantitatively and qualitatively identical with normal serum albumin in Dale tests. (4) Preliminary tests showed serum albumin dodecyl sulfate 4 to 8 times more potent as a sensitizing agent than normal serum albumin.

FACTORS AFFECTING ANAPHYLAXIS — Studies were continued to correlate antibody production with gross anaphylactic sensitivity and with sensitivity of excised smooth muscle. Incomplete evidence permit these observations. When guinea pigs are sensitized with small doses of ovalbumin, gross anaphylactic sensitivity first appears between the 8th and 11th day after sensitization, reaches a maximum on about the 14th day, and then decreases precipitously until the 21st to 28th day. Thereafter gross anaphylactic sensitivity increases slightly to a level which is maintained for the balance of the experimental period — 32 weeks. Antibody concentration in the serum of these sensitized animals reaches a maximum at about the 21st to 28th day — the period of lowest gross sensitivity — and then decreases to a slightly lower level where it is maintained for the balance of the experimental period. Thus, there is a definite inverse

relationship between antibody concentration in the blood and anaphylactic sensitivity of excised smooth muscle tissue. When guinea pigs are sensitized with large doses of ovalbumin, gross sensitivity first appears at about the same time as with small sensitizing doses, but the maximum degree of sensitivity attained is lower and appears a few days later. Gross sensitivity in these animals diminishes to a lower sustained level than in animals sensitized with small doses. In these animals also, there was an inverse relationship between antibody concentration and gross sensitivity and a direct relationship between tissue sensitivity and antibody concentration. When alum-precipitated ovalbumin was used as a sensitizing agent, gross anaphylactic sensitivity appeared at about the same time as with plain ovalbumin; the peak of maximum sensitivity as well as the abrupt decline in sensitivity appeared also at about the same rate, but the level of sustained sensitivity was much lower than in animals sensitized with plain ovalbumin. In these animals the antibody concentration increased to a maximum and did not diminish during the experimental period. Here again there was an inverse relationship between antibody concentration and gross sensitivity and a direct relationship between tissue sensitivity and antibody concentration.

INVESTIGATION OF ALLERGENS OF JOHNIN AND TUBERCULIN — Significance of poor growth of cultures used for production of tuberculin was investigated to determine whether a low yield of bacteria was due to limited bacterial multiplication or to lysis of the bacterial cells. Culture filtrates from poor-growth a nd normal-growth cultures were subjected to fractionation by procedures previously shown to concentrate the tuberculin. Yield of tuberculin from poor-growth culture filtrate was about one-third the yield from normal-growth cultures. Bovine skin tests of the two tuberculin fractions showed that both were more potent than Regular Tuberculin. Thus, the low yield of tuberculin from poor-growth cultures was not due to excessive lysis, but was the result of abnormally low rate of bacterial multiplication.

Fractionation studies were continued with both tuberculin and johnin. Countercurrent extraction of tuberculin culture filtrates with phenol was found to concentrate the tuberculin in the phenol fraction, but loss of active material was large.

ANALYSIS OF AMINO ACIDS AND PEPTIDES — Beginning study of the effect of enzymes on allergens showed serious limitations in available methods for following the successive stages in the enzymatic hydrolysis of proteins. A simple, rapid and accurate method for quantitative determination of peptides and amino acids was devised on the basis of photometric analysis of copper complexes of these hydrolytic products. Satisfactory application of the method was demonstrated by tracing the progressive hydrolysis of casein. Incidental to this study an ultraviolet absorption peak characteristic of solutions of the copper salts of amino acids and peptides was discovered. Absorption in the ultraviolet spectrum was so intense that a method over 100 times more sensitive than that based on measurement of the color of the copper salts of the amino acids appears possible.